ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE

Fiscal Year 1975

Volume I

(Parts I and II)

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

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(Parts I and II)

U.S. NATIONAL CANCER INSTITUTE

" Report of program activities.

Part I - Office of the Director

Part II - Division of Cancer Biology and Diagnosis

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1974 through June 30, 1975

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

Introduction

Shortly after the beginning of the Fiscal Year 1975, the National Cancer Institute began operations under the provisions of Public Law 93-352, the 1974 Amendments to the National Cancer Act of 1971 (Public Law 92-218).

During the year the following organizational changes were put into effect:

The Cancer Control Program was elevated to division status with the establishment of the Division of Cancer Control and Rehabilitation. Simultaneously with this action the Office of the Associate Director for Cancer Control was abolished. This new Division has the responsibility for planning and developing the cancer control programs authorized by the National Cancer Act of 1971 (P.L. 92-218). The Division also has the responsibility for planning and administering a research program for rehabilitation of the cancer patient. The Division operates through three major program areas. The Program Support area provides planning and evaluation support, resource development and communications and liaison support for the whole cancer control program. The Intervention Activities area develops and manages projects to demonstrate new or improved cancer intervention techniques in cancer prevention, detection, diagnosis, treatment, continuing care and rehabilitation. The Community Saturation area attempts to mobilize the full resources of a community to combat selected organ site cancers.

The Surgery Branch and the Radiation Oncology Branch were transferred from the Division of Cancer Biology and Diagnosis to the Division of Cancer Treatment. This move consolidated the three major modalities of treatment in a single organization and should facilitate NCI research in combined modalities of treatment. At the same time the position of Clinical Director was reassigned from the Office of the Director, NCI to the Division of Cancer Treatment. To complete this realignment of treatment activities, the title of the Medical Oncology Program was changed to Clinical Oncology Program to reflect the inclusion of other treatment modalities in addition to chemotherapy.

An Office of the Associate Director for Manpower Development was established in the Division of Cancer Research Resources and Centers to administer the various grant-supported training programs of the NCI. A Training Branch was established to administer fellowships and research training grants. An Education Branch will administer the newly developed Clinical Cancer Education grants.

The Laboratory of Immunodiagnosis was established in the Division of Cancer Biology and Diagnosis. This laboratory adds an intramural capability for research on cancer diagnosis in the Immunology Program.

Staff Changes, Activities, Honors and Awards

Dr. Maurice L. Simmons was appointed Director of Animal Care, OD

Dr. George P. Canellos was appointed Acting Clinical Director, OD

Dr. William E. Barkley was appointed Director of Research Safety, OD

Mrs. Elizabeth W. Stroud was appointed Personnel Officer, OD

Mr. James Graalman was appointed Chief, Research Contracts Branch, OD

Dr. Robert Love was appointed Chief, Program Analysis & Formulation Branch, OD

Mr. J. Paul Van Nevel was appointed Acting Associate Director for Cancer Communications, OD

Dr. Alan S. Rabson was appointed Director, Division of Cancer Biology & Diagnosis, DCBD

Dr. Paul P. Carbone was appointed Special Assistant to the Director, DCBD

Dr. Maxine F. Singer was appointed Chief, Nucleic Acid & Enzymology Section, DCBD

Dr. Ronald Herberman was appointed Laboratory Chief, Immunodiagnosis, Immunology Branch, DCBD

Dr. Jane Taylor was appointed Acting Chief, Breast Cancer Coordinating Branch, DCBD

Dr. Steven A. Rosenberg was appointed Chief, Surgery Branch, DCT

Dr. Vincent T. DeVita, Jr. was appointed Director, Division of Cancer Treatment

Dr. Stephen Carter was appointed Deputy Director, Division of Cancer Treatment

Mr. Philip Amoruso was appointed Administrative Officer, DCT

Dr. Gary W. Flamm was appointed Assistant Director, DCCP

Dr. Robert Squire was appointed Associate Chief, Experimental Pathology Branch, DCCP

Dr. Thomas J. King was appointed Acting Director, Division of Cancer Research Resources & Centers

Dr. Mary Fink was appointed Acting Associate Director for Research Programs, DCRRC

DHEW Superior Service Awards

Mr. Richard L. Sherbert, Chief, Management Policy Branch, NCI

Dr. Diane J. Fink, Director, Division of Cancer Control and Rehabilitation

Dr. Guy R. Newell, Deputy Director, National Cancer Institute

Dr. Robert C. Gallo, Chief, Laboratory of Tumor Cell Biology, Experimental Therapeutics, Division of Cancer Treatment, NCI

Dr. Samuel Price, Chief, National Organ Site Programs Branch

Dr. Vincent T. DeVita, Jr., Director, Division of Cancer Treatment

Dr. Maxine F. Singer, Head, Nucleic Acids Enzymology Section

Mr. Charles E. Leasure, Jr., Administrative Officer, Division of Cancer Treatment

DHEW Distinguished Service Award

Dr. Frank J. Rauscher, Jr., Director, National Cancer Program, National Cancer Institute

Other Honors and Awards

Dr. William A. Walter, Jr., Deputy Director, DCRRC, NCI received his second PHS Commendation Medal

Dr. Robert C. Gallo, Chief, Laboratory of Tumor Cell Biology, NCI, received the honorary Sc.D. from Providence College

Dr. Alfred S. Ketcham, Clinical Director and Chief, Surgery Branch, NCI, was awarded the Thomas E. Raffington Memorial Professorship in Clinical Oncology at the University of Miami School of Medicine

Dr. Joseph F. Saunders, Deputy Associate Director for International Affairs, NCI, was one of the recipients of the Group Achievement Award presented to the M212 Biocore Experimental Team by NASA

Dr. George J. Todaro, Chief of the Viral Leukemia and Lymphoma Branch, NCI, was honored by the American Society for Experimental Pathology as "the member under 40 years of age who has made the most outstanding contribution to the conquest of disease during the past year."

Program Activities Report
Fiscal Year 1974-75
Editorial Office of the National Cancer Institute

The Board of Editors reviewed 1,764 items for publication in various journals. Those not destined for the Journal of the National Cancer Institute constituted 746 manuscripts and 233 abstracts and book reviews.

Of 785 manuscripts submitted for publication in the Journal of the National Cancer Institute, 89 were by NCI authors: 56 accepted; 6 rejected; and 27 pending. Authors from other research institutions submitted 696: 283 accepted; 139 rejected; and 274 pending or withdrawn by author. Of these, 215 were received from other countries: Africa, Argentina, Australia, Belgium, Canada, China, Czechoslovakia, England, Finland, France, Germany, Hungary, India, Ireland, Israel, Italy, Japan, Nigeria, The Netherlands, Norway, Poland, Russia, Scotland, South Africa, Sweden, Switzerland, West Germany, and Yugoslavia.

Volumes 52 and 53 of the JNCI totaled 3,825 double-column pages.

Two NCI Monographs are now in press. NCI Monograph No. 41 ("Third National Cancer Survey: Incidence Data") totals 450 pages; it will be dated March 1975, but delivery is not expected until June or July 1975. NCI Monograph No. 42 ("Symposium on Ovarian Carcinoma") has been estimated to total approximately 180 printed pages; the copy of this monograph will be sent to GPO for printing the first part of June 1975.

1974

ANNUAL REPORT TO NIH

Office of International Affairs

National Cancer Institute

INTRODUCTION

The programmatic thrusts of the Office of International Affairs, within the Office of the Director, National Cancer Institute, evolve from those international activities which will contribute, ultimately, to the goals of the National Cancer Program. The mandate for the NCI, as specified in the National Cancer Act of 1971 and amended in 1974, is to provide a focal point for a multidisciplinary, concerted research effort against the world-wide problem posed by cancer. Accordingly, the international activities of the NCI include: (1) the support of research in the cancer field outside of the United States by highly qualified foreign nationals; (2) the support of collaborative research involving American and foreign participants; (3) the development and implementation of an International Cancer Research Data Bank; (4) maintenance of liaison and research collaboration with international agencies which have well-defined objectives and research programs in the cancer field; and (5) the support of training of American scientists abroad and foreign scientists in the United States.

During 1974, a major effort has been devoted to developing cohesive mechanisms for utilizing the cancer expertise that exists outside the United States. Increasing emphasis had been placed on expanding cooperation between the principle international organizations, institutions and personalities actively pursuing the scientific battle against cancer. Projects were established to accumulate as much information and to disseminate it, anticipating that it will serve as the data and information base for developing a comprehensive international program of collaboration in cancer research. Finally, measures were taken toward continuous improvement of international coordination of categoric research--basic, environmental and clinical--so that duplication of effort is avoided or minimized and that a "coupling" is facilitated between fundamental information and its immediate application to the clinical management of the cancer disease process in the patient. The highlights and milestones of these activities are described in the following sections.

THE INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) PROGRAM

The ICRDB Program has been developed in response to the mandate within the National Cancer Act of 1971 which states that the Director, NCI, shall:

> "Collect, analyze and disseminate all data useful in the prevention, diagnosis and treatment of cancer, including the establishment of an international cancer research data bank to collect, catalog, store and disseminate insofar as feasible the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country."

Accordingly, the Director, NCI, is authorized:

> "To take necessary action to insure that all channels for the dissemination and exchange of scientific knowledge and information are maintained between the National Cancer Institute and the other scientific, medical and biomedical disciplines and organizations, nationally and internationally."

In pursuit of these objectives and goals, a detailed plan for the ICRDB Program had been prepared and during 1974 the program became operational with the implementation of an on-line system called CANCERLINE (Cancer Information on Line) developed jointly with the National Library of Medicine.

Domestically and internationally, the ICRDB Program effort is dedicated to active promotion and facilitation, on a world-wide basis, of an exchange of information between cancer scientists and the dissemination of information through cancer centers and appropriate organizations to physicians for the care of the cancer patient.

35,000 cancer therapy and carcinogenesis abstracts are now available, through CANCERLINE, for on-line searching at more than 400 locations throughout the United States and abroad via terminals linked to the computer network of the National Library of Medicine. A procurement has been initiated for the rapid screening of more than 1,000 scientific journals for cancer-related articles from which it is estimated that approximately 4,000 abstracts will be prepared annually. These in addition to the abstracts prepared each year on cancer therapy and carcinogenesis, will result in an annual CANCERLINE growth rate of 15,000 abstracts.

During 1974, an interagency agreement was signed with the Smithsonian Science Information Exchange to establish a Current Cancer Research Project Analysis Center (CCRESPAC). This Center is collecting approximately 10,000 descriptions of ongoing, individual cancer research projects, including brief outlines of cancer therapy protocols. These project summaries from this Center will be available for on-line searching throughout the Unite States. Additionally, some 30 technical bulletins will be prepared for listing current research in specific subject areas for distribution to those scientists working in the same or similar scientific area for more direct and expedient communication with each other.

Procurements have been implemented for the establishment of four Cancer Information Dissemination and Analysis Centers (CIDACs). The objectives of each CIDAC include: (1) a steady stream of abstracts in specific subject areas sent automatically to scientists actively engaged in these areas; and (2) the publication of monographs on these specific areas from information available in CANCERLINE. CIDACs will be established in the following four subject areas: (1) Detection, Diagnosis, and Therapy; (2) Chemical, Environmental and Physical Carcinogenesis; (3) Cancer Virology; and (4) Immunology, Biochemistry and Cell Biology. A Computer Support Contractor will assist the CIDACs in disseminating current awareness services, for publishing technical bulletins, in conducting retrospective searches, and, for developing specialized products and services required by selected users.

Internationally, the ICRDB Program has included the support of national and international groups for the improvement of methods for handling clinical cancer data. For example, an International Tumor Immunotherapy Registry project resulted in the collection and analysis of nearly 160 clinical tumor immunotherapy protocols. Included in this endeavor is the support of meetings of scientists from major cancer centers for the establishment of a common clinical cancer data base.

The ICRDB Program in cooperation with the International Agency for Research on Cancer (Lyon, France) and the Center for Cancer Research (Heidelberg, Germany), sponsors an International Cancer Epidemiology Clearing House. Detailed data is collected, processed and disseminated to the scientific community on research related to cancer epidemiology and studies of causes of human cancer in countries throughout the world.

The World Health Organization receives ICRDB Program support for its effort on the International Classification of Diseases in Oncology in anticipation of an improved method of coding clinical cancer data.

Through the activities of a special Committee for International Collaborative Activities within the framewrok of the International Union Against Cancer, the ICRDB Program is assisted in the collection of data on on-going cancer research projects from countries around the world. Included are the identification and promotion of collaborative projects between cancer centers and cancer scientists in different countries. The International Union has also compiled a preliminary directory containing basic information on the world cancer centers.

COOPERATIVE ACTIVITIES

Cooperative cancer programs have been established with a number of foreign nations either as bilateral agreements between governments or formal agreements between the National Cancer Institute and a Scientific Institute or Scientific Organization of a foreign country.

As provided under the 23 May 1972 US-USSR Agreement for Health Cooperation, joint activity is underway in six major problem areas: (1) cancer chemotherapy; (2) the immunotherapy of human tumors; (3) the study of leukemia and tumor viruses of animals and man; (4) mammalian somatic cell genetics related to neoplasia; (5) the epidemiology of cancer; and (6) cancer control programs and cancer center organization, function and scientific and clinical effort.

In the area of chemotherapy, two Soviet drugs--FTORAFUR and ASALEY--have been tried clinically on patients in the M.D. Anderson Hospital and Tumor Institute, Houston, Texas. In 55 patients, ASALEY, exhibited an 18% remission rate in cancer patients with melanoma, myeloma, and colorectal cancer. FTORAFUR demonstrated an efficacy equal to the U.S. drug

5-fluorouracil, but without the side effects of stomatitis and myelosuppression. In 16 patients with adenocarcinoma of the gastrointestinal tract, 4 or 25% showed remission of the disease. Because of its lack of significant toxicity, FTORAFUR is considered to be a compound that is ideal for combination chemotherapy. A comparative study with 5-fluorouracil in a multiple drug combination for adenocarcinoma of the G.I. tract is in progress.

The results of experimental studies of American and Soviet drugs, that had been exchanged, were impressive and important progress has been achieved. During the third meeting of the US-USSR Joint Working Group for Cancer Chemotherapy, it was considered that the exchange of drugs has been most profitable to both sides. Agreement was reached to continue the search for new drugs as well as effective combinations of presently exchanged drugs for use in the treatment of advanced tumors.

Within the framework of American/Soviet cooperation, an international conference was held in September, 1974 in Geneva on "Screening Methodology for Antineoplastic Drugs." Under the aegis of the World Health Organization, the conferees attempted to standardize the screening of anti-cancer drugs so that results can be universally interpreted.

During the second meeting of the US-USSR Joint Working Group for the Immunotherapy of Cancer recognized a major breakthrough in the treatment of metastic malignant melanoma. Using at least three courses of the combination of three chemotherapeutic agents plus BCG (immunotherapeutic agent), 14 of 16 patients demonstrated an effective therapetuic effect. Five patients showed a 50% decrease and 6 had a 25% regression in the disease process whereas 3 patients showed disease stabilization. In general, the results were impressive, in that large numbers of patients had been admitted to the USSR protocols in a relatively short period of time.

Specific antigens have been prepared from primary melanoma tumor cells for studying the immune response of melanoma patients and diagnosis of the disease. These antigens used by A. Hollinshead (USA) are similar to those used by V. Gorodilova (USSR) for therapeutic purposes. The antigens are being compared, biochemically, as part of a cooperative study in progress by the two scientists.

Members of the US-USSR Joint Working Group on Cancer Virology presented results of their recent studies during a symposium held as part of the third meeting of the group at NCI. Progress was considered satisfactory on specific findings on: (1) cell lines from human cancer patients; (2) further characterization of the group of viruses found in these cell lines; (3) isolation and characterization of type C viruses and herpes-viruses from non-human primates with and without induced neoplasms; and (4) immunochemical properties of proteins from mouse type C viruses and the antisera produced against them.

During the second meeting of the US-USSR Joint Working Group for the Epidemiology of Cancer, American and Soviet scientists continued their exchange of information on the status of scientific investigations in cancer epidemiology in the U.S. and the USSR. Data were presented for recent years, which characterized specific features of cancer pattern distribution within the two countries. Both delegations reaffirmed that, in studying cancer epidemiology in various regions and among various ethnic groups, the main task consists of evaluating the role of external and internal factors in cancer morbidity and mortality. Two major agreements were reached as a result of the information exchanged on deliberated problems. First, a monograph will be published jointly on the current state of cancer epidemiology research in the US and the USSR giving recent data on cancer incidence, mortality, treatment and survival. Secondly, joint epidemiologic research on breast cancer will be undertaken by the NCI and its consultants and the Institute of Experimental and Clinical Oncology, Moscow, USSR.

In December, 1974, the NCI sent a delegation of six to the Soviet Union for the exchange of information on the status of biomedical instrumentation in the two countries. Of prime interest to the delegation were developments in culposcopy, miniaturization of mass spectrometric equipment and remote delivery of radioactive isotopes for treatment of colo-rectal and gynecologic cancers. Four Soviet delegations had come to the United States: (1) Cancer Epidemiology--5 members; (2) Cancer Chemotherapy--4 members; (3) Cancer Immunotherapy--5 members; and (4) Cancer Virology--4 members.

Three individual U.S. scientists visited the Soviet Union during 1974. An epidemiologist--cancer registry type and a geneticist-virologist spent three weeks each in various USSR institutes exchanging information in their respective specialties. The third, an epidemiologist, went with the intention of spending one year in the Soviet Union. The NCI has hosted a Soviet thoracic surgeon-chemotherapist (two months), two leukemia specialists (six weeks, each), three immunologists-immunotherapists (each for a month), a pediatric oncologist (two months), a rehabilitation specialist (two months), a specialist in cell separation techniques (two months) and two clinical pharmacologists (one month each).

As a result of the 15 May 1974 Agreement reached between the National Cancer Institute and the Japan Society for the Promotion of Science, eleven project areas were selected for joint study. Thus, US-Japan Cooperation in Cancer Research includes program development and implementation in chemical carcinogenesis, cancer virology, cancer immunology, cancer therapy, analytic epidemiology, breast cancer, lung cancer, bladder cancer, high energy radiation therapy, cytology and metastasis. Specified in the agreement between the agencies are: (1) joint sponsorship of selected scientific seminars to facilitate the exchange of information on the status of cancer research in the U.S. and Japan; (2) the support of the exchange of scientists between the two countries for short and protracted visits for collaborative research; and (3) the promotion of an exchange of materials such as drugs and biologics for experimental and clinical evaluation and utility.

During the first meeting of the US-Japan Joint Working Group on Cancer Therapy, a symposium was featured on "The Comparative Study on Cytosine Arabinoside and Cyclocytidine." A review of the experimental and clinical studies by both groups indicates that cytosine arabinoside (AraC), an American drug, is more effective than cyclocytidine (a Japanese drug) in the treatment of acute myelogenous leukemia showing, in addition, a lower level of toxicity. Agreement was reached to conduct: (1) cooperative studies with "new" drugs; (2) studies of specified cancers and their treatment such as gastrointestinal tumors; and (3) a symposium on the current status of nitrosoureas.

Plans have been reached by the US-Japan Joint Working Group on Cytology for standardization of procedures for evaluating cytologic tests; and an exchange of information on cytology of the pancreas, including new techniques for collecting material from the pancreatic duct as well as other special technical and clinical procedures. There was agreement to a continuing exchange of information on research and development in the field of cytology automation.

The Director of the National Cancer Institute and members of his staff met with a Delegation of scientists from the Federal Republic of Germany (FRG), representing the Ministry of Research and Technology (Bonn), the Society of Radiation and Environmental Research (Munich), and the Cancer Research Center (Heidelberg). The purpose of the mission was convincing expression of interest by the FRG in developing agreements for cooperation in research with specific U.S. agencies, in this case, the NCI.

The two areas of research of particular interest to both the Ministry of Research and Technology and the Society for Radiation and Environmental Research are: (1) instrumentation for early diagnosis including pattern recognition and isotope scanning; and (2) radiotherapy with particle emission.

After reasonable discussion, it became evident that there were several areas for U.S.-German cooperation in cancer research under the auspices of an agreement, to be formalized between DHEW and the FRG Ministry for Research and Technology. The first areas would relate to an exchange of scientists for research in automation of cytologic analysis, diagnostics, etc. The second could be an exchange of information on high energy particle radiation, including possible training of German scientists at Los Alamos. The third, and perhaps of more immediate interest would be NCI-FRG cooperation in the isolation, characterization and assessment of the clinical efficacy of extracts from plants from the Amazon, a cooperative project now in effect between NHLI and the FRG for finding potential cardiovascular agents.

During a meeting between the Director, NCI, and members of his staff with the Scientific Director for the National Institute of Health and Medical Research (INSERM), Dr. P. Laudat, reference was made to the 1974 Martinique meeting between Presidents Ford and Giscard d'Estaing of France. Dr. Laudat summarized the interests of the two Chiefs of State in furthering American-French Cooperation in Health, especially in cancer research and cancer control activities. There was a consensus that a formal agreement was not necessary, yet there was recognition of the pressures on both sides for something more prominent internationally than the existing NIH-INSERM Agreement. Consequently, it was agreed to establish a small US-France strategy group to establish means for effective cooperation which would include workshops on specific cancer problems and the exchange of scientists, information and research materials.

Representatives of the Polish Peoples Republic met with NCI personnel to learn of the NCI protracted plans for cooperation in cancer research sanctioned under the US-PPR Agreement for Cooperation in the Field of Public Health. The spokesman for the PPR Delegation indicated that the Polish government was most interested in an Institute-to-Institute relationship and that applied research and improvement of clinical resources were of highest priority in Poland. To-date there has been a fairly good exchange of information and materials. Five American scientists will visit Poland as part of the exchange and some ten Polish scientists will visit the U.S. Nevertheless, it was agreed that for the immediate future: (1) there should be an institute-to-institute agreement between the NCI and the Institute of Oncology, Warsaw which NCI, at this point, considers to be in effect; (2) the Director of the Warsaw counterpart of NCI will identify his cancer priority areas; (3) a cooperative program of interest to both sides should be established; and (4) to continue to exchange, in the interim, information, personnel and materials.

Finally, a delegation of biochemists and molecular biologists from the People's Republic of China were guests of the National Cancer Institute. The group's interests were oncogenic viruses; biochemistry of nucleic acids, enzymes and proteins; membrane biochemistry; and the applications of basic information derived from efforts in molecular biology and biophysics. An observation by NCI specialists in viral oncology led to agreement that there is very little research of this nature in the PRC and that a scientist-to-scientist interaction between US and PRC scientists could be of value.

COLLABORATIVE RESEARCH

Within the purview of the five operating divisions of the National Cancer Institute, 105 visiting scientists, representing 31 countries, were engaged in collaborative cancer research projects with senior NCI investigators. Of these, 23 guest workers received financial support from their own countries or other sources, while engaging in cancer research under the guidance of their NCI hosts, Table 1 and 2.

By means of grants and contracts outside of the United States, the NCI is in a position to increase communication among the world-wide scientific community and to maximize its opportunities to acquire important new knowledge as it evolves from national and international laboratories. In pursuit of this goal, the operating divisions of NCI support various projects in foreign countries in which there are unique facilities and resources available and outstanding capabilities and expertise among their scientists. Twenty-six grants have been awarded to scientists in 22 institutions in 14 different countries. Eighty-nine contracts are active in institutions of 19 countries. These are summarized in Tables 1 and 2. The thrust of the research supported by this estimated financial support of approximately six and one-half million dollars is in the areas of carcinogenesis and cancer epidemiology; cancer biology; immunology and immunotherapy; cancer virology; chemotherapy and radiation therapy; leukemia and lymphoma as well as lung and breast cancer.

There are 10 active cancer research projects being funded under the NIH Special Foreign Currency (PL 480) Program. Six of these are in India, and one each in Israel, Poland, Tunisia and Yugoslavia. In India three projects deal with the problem of cancer of the oral cavity and associated oral conditions. The other three projects are related to cancer chemotherapy including the screening of indigenous herbal drugs and other substances of plant origin. The Israeli research deals with the screening, isolation and characterization of plant substances for anti-tumor activity. In Poland, the epidemiology of cancer is being explored and analyzed, in

Table 1. GUEST WORKERS AT NCI (1974)

1.	Belgium	2
2.	Canada	1
3.	China	1
4.	Colombia	1
5.	Czechoslovakia	1
6.	France	3
7.	Germany (West)	2
8.	Greece	1
9.	India	1
10.	Israel	1
11.	Italy	1
12.	Japan	5
13.	Korea	1
14.	Malaysia	1
15.	Norway	1
	TOTAL	23

Table 2. NCI VISITING SCIENTISTS PROGRAM

		1974	1975
1.	Argentina	1	2
2.	Australia	3	3
3.	Belgium	3	3
4.	Canada	2	2
5.	Chile	1	1
6.	China (Taiwan)	3	5
7.	Czechoslovakia	1	1
8.	Denmark	0	1
9.	Finland	1	1
10.	France	4	5
11.	Greece	1	1
12.	Hong Kong	2	2
13.	India	12	19
14.	Israel	6	8
15.	Italy	6	7
16.	Japan	15	16
17.	Jordan	1	1
18.	Korea	1	2
19.	Netherlands	2	3
20.	New Zealand	0	1
21.	Pakistan	1	1
22.	Peru	1	1
23.	Philippines	1	1
24.	Poland	1	2
25.	Spain	1	1
26.	Sweden	0	1
27.	Switzerland	2	3
28.	United Kingdom	7	7
29.	West Germany	3	3
	TOTAL	82	104

Tunisia it is inflammatory breast cancer, and the effort in Yugoslavia is directed toward the study of immunosuppressive and anti-leukemic activity of pharmacologic agents. It is estimated that approximately one-half million equivalent U.S. dollars are expended for the support of this research.

COLLABORATION WITH INTERNATIONAL ORGANIZATIONS

Cooperation with the Pan American Health Organization (PAHO) in the development of a Latin American Cancer Research Information Program is progressing well. Two purchase orders have been provided to PAHO for the support of this program. One of the products of this effort will be a directory which will identify and describe comprehensive cancer research institutes as well as university departments and biomedical research centers with structured programs of cancer research. In addition, the directory would identify in each Latin American country the focus of cancer research activity. Another major end-point in the NCI-PAHO agreement is the collection of on-going research information for inclusion in the International Cancer Research Data Bank.

Table 3. NCI INTERNATIONAL GRANTS AND CONTRACTS

	CONTRACTS	GRANTS	COUNTRIES	INSTITUTIONS	PRINCIPAL SCIENTISTS
Division of Cancer Biology and Diagnosis	36		10	18	32
Division of Cancer Cause and Prevention	38		13	27	38
Division of Cancer Treatment	15		10	13	15
Division of Cancer Research Resources and Centers		26	14	22	26

Table 4. INTERNATIONAL DISTRIBUTION OF NCI GRANTS AND CONTRACTS

	CONTRACTS	GRANTS	INSTITUTIONS	PRINCIPA SCIENTIS
Australia	5	2	3	7
Austria	1		1	1
Belgium	1	1	2	2
Canada	8	5	11	12
Columbia	1		1	1
Denmark		1	1	1
Finland	4	1	2	5
France	8	2	5	10
Israel	20	2	5	21
Italy	6	2	5	8
Japan	7	1	6	8
Netherlands	5		3	5
Norway	1		1	1
Portugal		1	1	1
South Africa	1		1	1
Spain	1	1	2	2
Sweden	11	2	4	13
Switzerland	1	2	2	3
Uganda	1		1	1
United Kingdom	6	3	7	9
West Germany	1		1	1
TOTALS	89	26	65	113

Interagency Agreement: Smithsonian Institution (SI)

Title: Establish and Operate a Current Cancer Research Project Analysis Center (CCRESPAC)

Contractor's Project Director: Dr. Willis Foster, M.D.

Project Officer: Richard H. Amacher

Objective:

The overall objective of this Agreement is for the Smithsonian Institution to establish a Current Cancer Research Project Analysis Center (CCRESPAC) to support the International Cancer Research Data Bank (ICRDB) Program by processing research project descriptions specifying who is doing the research, where it is being done and what approach is being used.

Major Accomplishments:

- Prepared pilot file of approximately 200 cancer treatment protocols for future experimental work using CANCERLINE.
- Prepared an initial broad set of subject area definitions to categorize six specialized cancer catalog volumes for distribution to investigators and organizations working in these areas and those that support or perform research on cancer-related problems.
- Forwarded correspondence to several countries as well as Cancer Center Directors for the establishment of input channels for current cancer research project descriptions.
- Produced an initial frequency list of cancer terminology to be used in developing a thesaurus of cancer terms.
- Categorized over 9,000 ongoing cancer research resumes into six specialized cancer catalogs and developed a subject index for same. Will have completed the computer tapes for Linotron printing of the catalogs by the middle of June.

Significance to the National Cancer Program:

In consonance with the National Cancer Act of 1971, this interagency agreement has given the ICRDB Program the cost savings benefit of using an existing organization with capabilities to collect, process, and disseminate ongoing cancer research project information to researchers anywhere. Support of this

Center will result in several products and services including the dissemination of specialized cancer catalogs and the on-line searching of current research project information.

Proposed Course:

Plans call for the continuation of this Agreement through December 30, 1977

Date Agreement Initiated: December 30, 1974

Current Agreement Level: $ 372,667

Interagency Agreement: National Library of Medicine (NLM)

Title: Establish and Operate an On-Line Cancer Information System CANCERLINE

Contractor's Project Director: Dr. Henry M. Kissman

Project Director: Dr. John H. Schneider

Objective:

The objective of this agrement is for the placement of cancer-related information (abstracts, citations, project descriptions, etc.) into data bases generated, maintained and operated on the NLM's computer systems, and for the dissemination of the data so placed to institutions via the NLM telecommunications network or via direct mailing of necessary tapes.

Major Accomplishments:

- Converted data received from NCI to a format agreed upon by NLM/NCI and made the data available to existing and new users of the NLM System through the NLM telecommunications network.
- Provided computer time and equipment for processing, storing and maintaining cacer therapy and carcinogenesis abstracts.
- Provided personnel to assist the NCI in the areas of programming, user assistance and training for CANCERLINE users.
- Provided NCI with basic statistics regarding the use of CANCERLINE.
- Converted the CANCERLINE data base from the ELHILL-2 to the ELHILL-3 system.
- Provided a work station for NCI staff person assigned to the NLM and temporary space for CANCERLINE training activities.
- Made CANCERLINE available to designated NCI users.

Significance to the National Cancer Program:

In consonance with the National Cancer Act of 1971, this interagency agreement has given the ICRDB Program the cost savings benefit of using an existing organization with capabilities to reformat, process, and make the results of cancer research available to more than 400 locations throughout the world via an existing telecommunications network resident at NLM. These locations include medical schools, medical research institutions, regional medical libraries and hospitals throughout the United States, and in several countries outside the U.S.

Proposed Course: Plans call for the continuation of this agreement through June 30, 1976

Date Agreement Initiated: July 1, 1974

Current Agreement Level: $ 350,000

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1974 through June 30, 1975

RESEARCH CONTRACTS BRANCH

General

Fiscal Year 1975 produced a continued growth in research contracting at NCI. The total amount of Research Contracts in FY 1975 reached $270 million (estimated), compared with $213 million in FY 1974, for an increase of 26.8%. This represented a continuation of a long-term trend, with the amount of such contracts rising from earlier levels of $78 million in FY 1971, $126 million in 1972, and $144 million in FY 1973. In the period 1971 through 1975 there has been a 3.5-fold increase in research contracts value at NCI. The numbers of such contracts rose from 445 in FY 1971 to 1,123 in FY 1975. These data are drawn from the accompanying table entitled "Research Contracts Awarded, NCI by Dollars and Numbers."

These rapid increases were related to the overall expansion of NCI's budget but also must be regarded as a recognition of the practical uses to which the contracts can be put in the attainment of the goals of a biomedical research program.

NCI's research contracts constitute the preponderance of NIH's research contracts workload. Thus, 77% of the NIH research contracts value of $349 million in FY 1975 consisted of NCI contracts.

Organization of RCB

RCB continued to follow its policy of adapting its organizational structure to the changing demands placed upon it. Whereas in FY 1973 it had initiated a new section in Cancer Control to handle contracts of that growing program, in FY 1974 it added two additional new sections in an area formerly handled by a single section dealing with the large contracts program of the Division of Cancer Cause and Prevention. The two newly established DCCP sections were the section for Viruses and Field Studies and Statistics, and the section for Carcinogenesis. These brought the total RCB Section to 5, the others being (1) Cancer Biology and Diagnosis, (2) Cancer Treatment, and (3) Cancer Control. These organizational additions enabled RCB to continue to implement its management philosophy of providing close-in support to the work of the operating divisions. Under this concept, Contracting Officers and Contract Specialists work in proximity to the separate Divisions of NCI, and thereby insure a smoothly working "team" approach to the Research Contracts work of NCI.

Distribution of Workload

An analysis of the detailed contracts workload data for NCI shows that the volume of work was substantial and growing in all Divisions in FY 1975. For detailed workload data see the accompanying table entitled "Research Contracts Awarded, NCI by Dollars and Numbers."

Frederick Cancer Research Center

The contract with Litton Bionetics to operate the Frederick Cancer Research Center at Ft. Detrick, initially awarded in June 1972, is one of the largest ever awarded by DHEW. It is growing, as indicated in the following table:

	Contract Award	Interdepartmental Support Agreement	Total
FY 1973	$10,645,000	$1,667,000	$12,312,000
FY 1974	$14,484,000	$2,800,000	$17,284,000
FY 1975	$21,913,000	$3,300,000	$25,213,000

In FY 1976, total expenditures are expected to exceed $25,600,000. The major policy development on this matter in FY 1974 was the recommendation of a committee of the National Cancer Advisory Board that there be appointed a Director of Research for the Facility to give greater attention to the basic research potential of the Center. A Search Committee was engaged in an effort to recruit and recommend for appointment to the Executive Committee such a Research Director. This recommendation was brought to fruition in February 1975 by the hiring of Dr. Michael Hanna. The basic research program operations budget for FY 1975 was $1.8 million and is estimated at $2.9 million for FY 1976.

The RCB staff at the FCRC consists of a Contracting Officer, a Contract Specialist, a part-time Contract Clerk, and a Stay-in-School typist position. The staff is concerned with providing expert advice to the other NCI representatives on contractual matters. Its activities include making "Contracting Officer Authorizations" as appropriate. There is a volume of 1000 "COA's" issued per year covering such matters as approval of contractor requests for Capital Equipment Acquisition, subcontracts, overtime work, etc. It also reviews the purchasing system of the contractor, and administers certain aspects of the award-fee reporting system. The principal activity is the negotiation and administration of the Cost-Plus-Award-Fee Contract.

EEO

During FY 1974 RCB established a position to coordinate contract compliance efforts of the NCI, its contractors and the Office of Civil Rights, DHEW. It also designed and put into effect for the use by its Contract Specialists, a Summary Information Sheet listing factors to be reviewed in ascertaining the extent of possible problem areas in EEO compliance by its contractors.

Project Officers of NCI are cooperating in this program, and consequently, with the Contract Specialists, are gaining greater familiarization with the EEO provisions of the contract.

Initial reaction to this program has been very favorable on the part of other Federal agencies and the affected contractors. The Office of Civil Rights, DHEW, in particular, endorsed this innovative program of NCI.

8A Program

During the past year, the Public Health Service set a goal of $700,000 for contracts to be awarded under Section 8A of the Small Business Act. RCB designated an 8A Program Coordinator who has actively pursued this end and we have awarded $323,000 (estimated) worth of 8A Contracts this year and hope to surpass the entire PHS goal next year.

Contracts Reports Unit

RCB acted as a coordinating point and staffed an NCI program in financial reporting of NCI contracts in FY 1974. A computerized monthly print-out was established of the financial status of each NCI contract by program area. This is a project which was initated by NCI, with programming and technical assistance from contractors. The resulting product is a management reporting system on contracts of benefit to NCI AO's and related program managers. It renders unnecessary much of the formerly tedious monthly financial reporting which had been centered in the separate NCI program offices under the responsibility of the AO's. This is regarded as a definite forward step in facilitating financial management of contracts at NCI and is continuously undergoing review with an eye toward improvement.

ANNUAL REPORT
RESEARCH CONTRACTS AWARDED NCI
BY DOLLARS AND NUMBERS

NCI RCB

Scientific Program Area	1971	1972	1973	1974	1975*
	(Dollars in thousands) (Years are Fiscal Years)				
Division of Cancer Cause & Prevention					
Office of Director	---	---	$ 615	$ 3,223	$ 6,773
(Numbers)	---	---	(1)	(13)	---
Carcinogenesis	$ 10,573	$ 23,214	27,547	29,649	34,203
(Numbers)	(71)	(125)	(122)	(169)	---
Virus	31,411	42,649	43,517	49,537	49,905
(Numbers)	(117)	(130)	(121)	(193)	---
Field Studies & Stat.	4,412	5,269	6,891	6,830	8,800
(Numbers)	(51)	(50)	(56)	(52)	---
Task Forces	---	---	---	---	---
Sub-Total	$ 46,396	$ 71,132	$ 78,570	$ 92,922	$104,806
(Numbers)	(239)	(305)	(300)	(427)	---
Division of Cancer Treatment	$ 24,235	$ 40,292	$ 42,255	$ 57,384	$ 61,868
(Numbers)	(165)	(202)	(227)	(277)	---
Task Forces	---	---	---	700	750
Sub-Total	---	---	---	$ 58,084	$ 62,618
Division of Cancer Bio. & Diag.	$ 4,354	$ 9,609	$ 11,717	$ 17,613	$ 22,799
(Numbers)	(40)	(74)	(114)	(265)	
Task Forces	---	---	---	7,881	8,510
Sub-Totals	---	---	---	$ 25,494	$ 31,309
Division of Cancer Control & Rehab.	---	---	$ 3,800	$ 26,912	$ 44,275
(Numbers)	---	---	(30)	(135)	---
Major Construction & Other	$ 3,000	$ 4,999	$ 8,000	$ 9,288	$ 26,585
(Numbers)	(1)	(7)	(6)	(19)	---
TOTALS	$ 77,985	$126,032	$144,342	$212,700	$269,593
(Numbers)	(445)	(588)	(677)	(1123)	---

*Data for 1975 estimated - Contract numbers cannot be estimated

ANNUAL REPORT
OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM PLANNING AND ANALYSIS (OADPPA)
NATIONAL CANCER INSTITUTE
July 1, 1974 - June 30, 1975

OFFICE OF THE ASSOCIATE DIRECTOR

The traditional functions of the OADPPA are providing leadership, consultation and direct participation in program analysis and planning as a service to NCI program units and to major non-Federal organizations participating in the cancer effort at the discretion of the Director of the National Cancer Program.

The office has major responsibility for the planning and analysis functions associated with the National Cancer Program (NCP) and the planning requirements of NIH and DHEW. The office coordinates activities for the preparation of the following:

(a) The annual plan for the NCP including 5-year budget projections for submission to the President,

(b) The NCP Strategic Plan,

(c) certain portions of the NIH Forward Plan,

(d) the NCI portion of the NIH Evaluation Plan,

(e) Operational Planning System (OPS) objectives for tracking by NIH and DHEW, and

(f) plans for individual research and control programs.

The office is also responsible for the development and implementation of the NCP Management Information System (MIS) in close collaboration with the NCI operating divisions.

Several personnel changes have taken place during the year. Dr. Robert Love, Professor of Pathology at the Thomas Jefferson University Medical College, Philadelphia, Pa. succeeded Dr. Abraham Cantarow as Chief, Program Analysis and Formulation Branch. Dr. Elliott Stonehill, Associate at the Sloan-Kettering Institute, New York, joined the Program Analysis and Formulation Branch as Research Planning Officer. Dr. Stonehill brings to the Branch a strong, research background in genetics, immunology, and molecular biology. Mrs. Barbara Murray formerly with the Division of Cancer Treatment joined the Systems Planning Branch and has assumed project responsibility for the development of the Management Information System.

The major accomplishments and activities of the OADPPA are described under the two Branches of the office. Although general assignments are given to each Branch, much of the work is accomplished by close collaboration through the mechanism of inter-branch teams for specific assignments.

PROGRAM ANALYSIS AND FORMULATION BRANCH

This Branch is concerned primarily with substantive scientific matters in cancer research and related activities. It prepares position papers outlining alternative approaches, guidelines and procedures and devises models for the review, analysis and implementation of Programs of the National Cancer Institute and National Cancer Plan. Members of this Branch participate in discussions, collaborate with the Systems Planning Branch, and prepare reports and other documents on progress toward major objectives and on identified needs of the Institute. These include analysis of current national programs, preparation of recommendations and options for initiation of major program modifications and the formulation and development of outlines for new programs.

The members of this Branch provide assistance to the Director, NCI, the Division Directors and Program Directors in matters relating to research planning, and program formulation as described, and to unique needs of the different divisions. Some examples include: the development of approaches, models and guidelines for formulating new research programs and for amending existing ones; responding to queries by extramural scientists concerning matters relating to the National Cancer Program that are referred to this Branch. It also maintains liaison with individuals and research groups outside the NCI on matters relevant to cancer research.

I. National Cancer Plan

During the past year, the PAFB has been engaged principally in activities concerned with the National Cancer Plan and related research projects supported by the NCP-NCI. These include:

A. Participation in preparation of the various required reports on the NCP Plan.

B. Completion of analysis of the FY 73 contract and grant supported projects of the National Cancer Institute with respect to the distribution of activities among the various areas of cancer research and the extent of coverage of the National Cancer Plan Project Areas and objectives by these projects.

C. Development and implementation of a contract with the TRW Systems Group to construct a computerized data base of scientific and administrative information concerning NCP Project Areas, grants and contracts. TRW has also developed computer programs to interrogate these data bases to allow analysis of the scientific content of projects and their association with Project Areas. The programs and data bases have been successfully used to produce numerous tables, data and reports specified by this Branch, e.g., research coverage in different areas of cancer, NCP Project Areas and Center grants.

D. Initiation and development of a contract with the Smithsonian Science Information Exchange for analysis of the subject content of fiscal year 1975 project grants and contracts awarded by NCI using the computerized data bases described.

E. Modification of the Project Analysis Form used to categorize the research content of FY 73 grants and contracts.

F. Negotiation of a contract to identify and assess the contribution of categorical biomedical research to basic science knowledge.

II. Publications, Presentation, and other Professional Activities

Influence of Drug Combination on Toxicity of Danomycin or Adriamycin. E.H. Herman and V.S. Waravdekar, paper presented at the XIth International Cancer Congress, held in Florence, Italy, October 1974.

SYSTEMS PLANNING BRANCH

This Branch is responsible for the development and application of systems planning techniques to cancer research and cancer control activities; providing direct support for National Cancer Program planning, department level planning, and individual program planning. The Branch also provides general planning consulting services to the various program areas within the National Cancer Institute, institutions and groups participating in the national program, and other institutes and groups within the NIH and the DHEW. In carrying out these responsibilities, the Branch staff participates as members of planning teams organized to develop individual program plans; works directly with program and administrative personnel in the development of operational plans; maintains liaison with program personnel; provides periodic consultation and direct efforts, as requested by program leaders to revise and update both program and operations plans; provides education and training to program staff in the use of systems techniques; works closely with the financial management staff during the budget preparation cycle to correlate budget preparation with existing plans.

Specific activities engaged in by the Branch during the past year are described in the paragraphs that follow:

I. National Cancer Program Planning Effort

A. Coordinated the development of the National Cancer Program Operational Plan which describes National Cancer Program content, revisions, distributions, and operating procedures. The plan was submitted to the President for transmittal to the Congress in September 1974.

B. Coordinated the preparation of the annual five-year National Cancer Plan as required by law for the five-year period FY 77 through FY 81 for submission to the President.

C. Initiated development of a capability to analyze and project resources requirements and to assist in planning for the acquisition of critical resources for the NCP. This capability permits consideration of resources at a gross level (i.e., overall level of manpower, facilities, supporting resources, and associated costs) or detailed level (e.g., manpower specialties) depending on the need. Efforts conducted to date have produced a data base reflecting resources as of FY 72 and two projection models; the Mid-Range Analysis Model which provided gross level projections for the NCP Strategic Plan, and a Programmatic Resources Model developed to provide detailed resources projections in support of the NCP Annual Plan and annual budget submissions.

D. A series of conferences to update the NCP scientific recommendations was held in 1973. The results of these conferences have been documented and work has been initiated to assess the impact of these revised recommendations on program content and future plans. Much of this effort has focused on enlisting the participation of NCI scientific staff with program responsibility in the process of formulating an updated program/strategy based on revised scientific recommendations.

II. Support of Department Level Planning

As the focal point for Department level planning, the Branch performed the following activities:

A. Prepared and monitored the NCI FY 75 DHEW Operational Planning System (OPS) objectives.

B. Coordinated the NCI DHEW Forward Planning activities.

C. Coordinated the NCI Evaluation Planning effort.

III. Research and Cancer Control Program Planning

In the preparation of specific research and control program plans, the Branch provided planning assistance and support to the following individual programs in NCI:

A. Breast Cancer Program

B. Prostate Cancer Program

C. Office of Cancer Communications

D. Division of Cancer Control and Rehabilitation

E. Cancer Centers Program

F. Carcinogenesis Program

G. Frederick Cancer Research Center

H. Cancer Treatment Program

IV. Systems Planning Consultation

As the focal point for development and application of systems planning techniques, the Branch engaged in the following activities:

A. Continued to provide assistance to the Division of Cancer Research Resources and Centers in developing scientific manpower and training requirements for the NCP.

B. Continued to provide assistance to the Division of Cancer Research Resources and Centers in developing facilities and construction requirements for the NCP.

C. Developed projections of NCI staffing requirements to support the NCP.

D. Continued to provide assistance to the Division of Cancer Cause and Prevention in the preparation of a long-range plan for the Frederick Cancer Research Center.

E. Provided assistance to the Division of Cancer Control and Rehabilitation in obtaining consensus judgements concerning relative program emphasis and allocation of resources among program activities.

The staff also participated in the evaluation of a number of planning and information services proposals both within the NCI and in other institutes of the NIH.

V. Management Information System

The initial implementation of selected subsystems mentioned in last year's report was continued; three subsystems were completed (two Operating Budgets Subsystems for the Financial Management Branch and the Division of Cancer Treatment/Contracts Management System Interface Subsystem for the Division of Cancer Treatment). Both have been in operation for a considerable time and are working well. Analysis was completed of two other subsystems (Drug/Disease for the Division of Cancer Treatment and the Scientific Indexing Subsystem for the Division of Cancer Research Resources and Centers).

The three subcommittees chartered by the MIS Advisory Group have completed their work, with the issuance of final reports. The Operations Subcommittee, in particular, has prepared extensive guidelines which will be helpful to those operating systems at the NCI as well as their larger contractors.

In August 1974, it became necessary to recompete the Phase II development of the MIS, and a new contract is anticipated shortly. In the interval, the MIS Project Office has directed its attention toward developing a MIS Strategy Report, redefining the information needs of the OD (a continuing, not a one-time operation) and developing reports which have been useful to the Executive Committee. These reports, prepared by NCI employees, have dealt with trends, exception reporting, and summary information of the type that appears useful at the OD level. Such sample reports have included a Director's Report (Summary of Personnel Actions); NCI Space Occupancy Analysis by Division; Committee Meeting Announcement System; and an NCI Personnel System linked to existing NIH and DHEW Data Systems.

Information systems have been developed for several divisions of the NCI. These systems contain information both in detail sufficient for the division operations, and in summarized form for the OD uses. It appears that such mechanisms, when available throughout the NCI, will provide an additional tool for the analysis of NCI programs across divisional lines.

VI. Presentations and Publications

The Systems Planning Branch staff has participated in or provided presentations on a variety of planning and analysis subject areas to interested organizations within the NIH. The office participated in the 1974 STEP series by providing two 1 day segments on program planning, resources allocation, forecasting techniques and program evaluation.

Publications

An Analysis of Mid-Range Resources Requirements for the National Cancer Program, NIH Publication.

Planning Biomedical Research Programs, NCI Publication, February 1974.

Systems Planning and Information Systems Overview. NCI Publication, February 1974.

Contract Narrative
Office of the Associate Director for Program
Planning and Analysis, NCI
FY 1975

Contractor: JRB Associates (Contract #NIH-NOI-CO-55426)

Title: Planning and Support Services for the National Cancer Program

Contractor's Project Director: Mr. John P. Weis

Project Officer: Jacqueline P. Parkman

Objective: Provide the support services necessary to assist NCI in meeting the expanded responsibilities established by the National Cancer Act of 1971.

Major Accomplishments: Provide planning support services for national and individual program planning activities.

Assist in the preparation of briefing and presentation materials.

Assisted in the preparation of draft documents required to develop the National Cancer Program Plan.

Provided administrative and logistical support for planning conferences and meetings.

Significance to the National Cancer Program: The expanded scope and responsibilities of the National Cancer Program has imposed additional requirements for reporting, planning and analyzing alternative courses of action. This contract provides assistance in areas which could not be done in NCI.

Proposed Course: This effort will be continued.

Date Contract Initiated: July 17, 1974

Current Contract Level: $2,100,000

Contract Narrative
Office of the Associate Director for Program Planning and Analysis, NCI
FY 1975

Contractor: JRB Associates (Contract #NIH-NOI-CO-45425)

Title: Resources Analysis and Planning for the NCP Budgeting and Implementation Support

Contractor's Project Director: Mr. Richard Danzeisen

Project Officer: Mr. Michael G. Brown

Objective: Develop and apply resources analysis and planning methodologies in support of National Cancer Program (NCP) planning and management.

Major Accomplishments: Update and continue development of a NCP resources data base and planning estimating relationships.

Development of a fiscal projection crosswalk model and application in support of the 1977-1981 NCP Annual Plan.

Continue development of cancer manpower projection methodologies.

Application of selected operations analysis techniques in support of individual program planning activities.

Studies to define approaches to developing priority information for NCP planning.

Significance to the National Cancer Program: The expanded scope and responsibilities of the NCP have imposed a need for a sound analytical basis for planning and decision-making. This contract is directed toward the development of the necessary analytical methodologies and techniques.

Proposed Course: This effort will be continued.

Date Contract Initiated: July 1, 1974

Current Contract Level: $660,000

Contract Narrative
Office of the Associate Director for Program
Planning and Analysis, NCI
FY 1975

Contractor: TRW Systems Group, Inc. (Contract #NIH-NO1-CO-55171)

Title: National Cancer Program Analysis System

Contractor's Project Director: Dr. John Miller

Project Officer: Joseph Sberro

Objective: To design and develop a methodology for analysis of the National Cancer Program Plan (NCPP) and of the NCI supported biomedical research activities. To provide specified reports of the analysis, plus an ad hoc query capability.

Major Accomplishments: The analytical methodology was translated into a computer system. The system was programmed to run on the DCRT installation. From information provided by PAFB/OPPA, a data base was created for the NCPP and for NCI grants and contracts.

The system was run to provide the specified reports for the NCI staff, and a number of ad hoc queries were processed.

The system design, program, and operation were fully documented.

Significance to the National Cancer Program: The analytical products will provide NCI with an objective measure of the coverage of the NCPP by NCI funded activities. The Plan and Program activities can then be brought into consonance. Specific questions about the NCPP and NCI activities can be answered from the data base.

Proposed Course: To up-date the information content based on recommended changes to the NCPP, and on new contracts and grants. To continue to enhance the system's analytical capability.

Date Contract Inititated: September 1974

Current Contract Level: $91,968

Contract Narrative
Office of the Associate Director for Program
Planning and Analysis, NCI
FY 1975

Contractor: The MITRE Corporation (Contract #NIH-NCI-73-5001)

Title: National Cancer Program Management System Planning and Analysis Information

Contractor's Project Director: Mr. Edward Sharp

Project Officer: Barbara Murray

Objective: To furnish technical information systems support services to the Office of the Director, NCI. Specifically, to provide technical assistance in the coordination of NCP-MIS development activities; the design, implementation and installation of the NCP-MIS in addition to preliminary analysis and study of critical short-term information requirements within the OD-NCI.

Major Accomplishments: Technical support was provided to develop an RFP for Phase II of the MIS as well as evaluation criteria. MITRE also assisted in compiling and documenting the award fee evaluation scores for the Phase I contract. The Operations Subcommittee of the MIS Advisory Committee received considerable assistance. The work culminated in a major report entitled National Cancer Program/Management Information System Operations Guidelines dated January 1975. The report presents the Operations Subcommittee's recommendation on guidelines for staffing, documentation, standards, data confidentiality and security procedures, data quality control, training and testing. MITRE Corporation is now completing a draft report reassessing the critical information needs of the OD, NCI and a Catalog of NCP/MIS Services.

Significance to the National Cancer Program: The National Cancer Act of 1971 provides for enhanced information systems throughout the Cancer Research Community. This contract gives the NCI the coordinating and evaluation support it needs to see that the NCI-MIS is successfully implemented and serves to achieve the goals of the NCP on technically sound and realistic approaches.

Proposed Course: This contract will be continued during design, development, integration and implementation of the MIS.

Date Contract Initiated: November 13, 1972

Current Contract Level: $560,000

Contract Narrative
Office of the Associate Director for Program
Planning and Analysis, NCI
FY 1975

Contractor: Smithsonian Institution (Contract #NIH-NCI-NO1-CO-55196)

Title: NCP Analysis of Subject Content of Fiscal Year 75 Grants and Contracts Awarded by the NCI

Contractor's Project Director: Dr. W.R. Foster

Project Officer: Dr. Robert Love

Objective: To obtain necessary grant applications and contract information from NCI and construct a working file for the hard copy documents. Make those available for manual use by the scientific staff as necessary. Analyze each document in accordance with the established procedures of the PAFB staff including the summary of administration and general classification data pertinent to the overall project, and the marking of subject-codes on appropriate records for use in the computer text processing system maintained for NCI. Deliver to NCI all material required for further processing of the finished analysis and text sentences.

Major Accomplishments: Work has not yet begun on this contract.

Significance to the National Cancer Program: This project directly supports the National Cancer Program Plan activity, which is the guiding framework for the attack on cancer. This work is relevant to the establishment and maintenance of program balance.

Proposed Course: The contract is planned for 3 years.

Date Contract Initiated: February 1, 1975

Current Contract Level: $35,000

Office of Cancer Communications

Office of the Director

National Cancer Institute

Program Activities Report, July 1, 1974--June 30, 1975

The Associate Director for Cancer Communications, Frank Karel III, resigned September 30 to join the Robert Wood Johnson Foundation and J. Paul Van Nevel, formerly Deputy Associate Director, was named Acting Associate Director. The Office consists of the Immediate Office of the Associate Director; Program Liaison Branch (Robert G. Schonfeld, Chief), and the Education and Technical Reports Branch (William S. Gray, Chief). Under the last-named entity are the Research and Program Reports Section and the Public Inquiries Section.

Mrs. Elaine Freeman, Head, Cancer Control Communications Unit, resigned to return to her former position at Johns Hopkins University. Mrs. Elaine Bratic came to the staff of the Office from the National Clearinghouse for Smoking and Health. Under the terms of the Intergovernmental Personnel Act, the staff was augmented by Roger Shaffer, from Ohio State University; Ms. Lorraine Kershner, from the University of California at Los Angeles; and Ms. Gay Allen, from the University of Louisville. Mrs. Bratic and Mr. Shaffer were assigned to assist the Director, Division of Cancer Control and Rehabilitation, in the communications effort. Ms. Kershner was assigned as a science writer in the Research and Program Reports Section, nd Ms. Allen, under the aegis of the Program Liaison Branch, is charged with the development of ETAC (Envoys to Talk About Cancer) an undertaking involving the initiation of an NCI speakers bureau and the booking of NCI staff on community television programs nationwide.

Also recruited as science writers for the staff of the Research and rogram Reports Section were Larry Blaser, formerly with a private scientific esearch firm, and Dennis Doris, who had been a reporter for International edical News.

Joan Hartman, of the University of Colorado, also joined the staff on he last day of the fiscal year, under the Intergovernmental Personnel Act. he will serve in the Program Liaison Branch.

A new development was a cancer control communications program, including comprehensive cancer centers communications network. This program is eing developed to make cancer knowledge and technology available to health ractitioners and the public, and to motivate them to use this knowledge and chnology. The approach taken by the communications program is to create climate of awareness within which specific programs and projects can be ccessfully introduced, conducted, and coordinated. The program will einforce and coordinate professional and public education at national, egional, and community levels. It will also seek to stimulate private and mmunity organizations to invest in coordinated educational activities.

The Comprehensive Cancer Centers, which are providing a national resource for clinical research, basic research, training, and demonstration of patient care, are expected to serve as focal points for a variety of community efforts. They will work to assure the widespread use of the best available methods for early detection, diagnosis, and treatment of cancer, collection of data useful in the prevention of cancer, and dissemination of information, both at the public and professional levels. Each Center's efforts to provide informational and educational programs will be supported by NCI through the communications network.

With the assistance of Biospherics, Incorporated, a private firm with which the Office of Cancer Communications has a contract for the processing of inquiries, a source book has been developed to aid communications personnel at the various centers in replying to questions about all aspects of the cancer problem.

The OCC also undertook the development of a national WATS telephone line, to back up similar lines NCI is establishing in the Comprehensive Centers. The WATS line is a system whereby any person in the continental United States may telephone a Center or NCI itself--free of charge--to obtain cancer information.

Also established by the OCC was a regular program of communications workshops for information personnel of the Centers, to give them the benefit of NCI expertise in the area of communicating information and increasing the awareness of the public about the progress and problems involved in the battle against malignant disease.

Public and Congressional Inquiries

Shortly before the beginning of the Fiscal Year, the OCC entered into a contract with Biospherics, Incorporated, of Rockville, Maryland to produce responses to written public and congressional inquiries received by the NCI on various aspects of cancer. These are divided into "custom" letters, i.e., those requiring individually typed and signed letters to specific individuals, discussing specific problems peculiar to the inquirer. "Non-custom" letters are those requesting literature about cancer, and can be replied to by simply sending the correspondent one or more booklets or pamphlets accompanied by a printed "thank you" card. When the contract was negotiated, it was expected that OCC would receive 5,000 custom and 16,000 non-custom inquiries. These figures turned out to be unrealistic, owing to vastly increased visibility of the NCI and the NCP. For the Fiscal Year the Office responded to 12,650 custom inquiries and 9,300 non-custom. A total of 59,000 NCI publications was mailed out, and responses were prepared to 325 inquiries from members of the Congress.

Inquiries covered the whole spectrum of the cancer problem--carcinogenic substances, proposed cancer cures, appropriations and funding, research grants and contracts, and cancer incidence and mortality figures. By far the greatest increase in inquiries, however, was due to persons in search of information concerning their own illnesses or those of members of their families. Information in the news media that OCC was prepared to offer advice on consultations to attending physicians of cancer patients was responsible in large measure for the increased influx of requests.

For example, the widely-read magazine Woman's Day ran a two-part story on cancer by Jane E. Brody, directing its readers to write OCC for cancer information and referral services. An article in the Sunday supplement, Parade, which appears in about 100 newspapers, offered similar directions. The National Observer followed suit in a front-page article by Patrick Young. Other publications which produced a flood of inquiries were the National Enquirer, which published a piece extolling the (unproven) Maruyama cancer vaccine from Japan; New York magazine, with an article, "The Politics of Cancer," criticizing NCI funding policies, and Midnight, which generated a torrent of mail with its article on the use of beagles in cigarette-smoking studies. The Canadian Broadcasting Company staged a television program on Laetrile, the thrust of which was that Sloan-Kettering Institute findings on the substance were being "suppressed," and viewers were encouraged to write to President's Cancer Panel Chairman Benno C. Schmidt. This resulted in some 500 letters which were turned over to OCC for response. Outside the media, the National Health Federation sponsored a mass-mailing campaign, charging that NCI's county-by-county survey of cancer mortality "proved" that drinking-water fluoridation was a cause of cancer.

In addition to mail replies to public inquiries, approximately 9,000 telephone queries from the general public and the Congress were handled by OCC staff during the year. The content of these questions generally followed that of the written inquiries, with cancer-patient questions outnumbering other types.

Publications and Exhibits

Twenty new and revised publications for the general public are being prepared under contract in cooperation with the American Cancer Society. The new series will consist of a core publication with general information about cancer cause, prevention, detection, diagnosis and treatment; a leaflet containing salient points from the core publication; and a series of eighteen pamphlets dealing with cancer of specific sites and systems: bladder, bone and connecting tissue, brain and central nervous system, breast, colon and rectum, female reproductive system, lung, head and neck, pancreas, male genito-urinary organs, skin, stomach and esophagus, leukemias, childhood leukemia, lymphomas, Hodgkin's disease, multiple myeloma, and malignant melanoma. These publications will be made available to the cancer centers and ACS divisions.

Ten thousand plastic-wrapped packages of five reports each (NCP Director's Report, Report of the National Cancer Advisory Board, Report of the President's Cancer Panel, etc.) were distributed to interested persons and cancer or cancer-related groups.

The NCI Publications and Journals exhibit was shown at meetings of the National Medical Association, the American Public Health Association, and the ACS-NCI National Conference on Advances in Cancer Management, Parts I and II.

A new exhibit was developed for the NIH Alumni Reunion and NIH Open House presenting historical highlights as well as the National Cancer Program. This exhibit will remain on display at NIH through the Bicentennial year. For the NIH Open House the following displays were presented:

1. In the Tent, a blood-cell separator and slide presentation by an NCI nurse.

2. In Building 37, drug development; continuous infusion of a rat; tissue culture apparatus.

3. In Building 10, Laminar Flow Room.

4. In Building 37, seven laboratories were open for visitors.

An estimated 30,000 people visited NIH during Open House.

During the year, 13 Special Communications from the Director, NCI, were mailed out.

Press Activities

OCC resumed the operation of news rooms at major scientific meetings, including the National Conference on Advances in Cancer Management, Part I in New York City and Part II in Denver, and the NCI Breast Cancer Task Force "Report to the Profession" in Bethesda.

OCC staff also assisted news science writers in covering NCI-sponsored conferences and workshops, including the Estrogen Receptor Clinical Correlation Workshop in Bethesda, the International Workshop on Cell Surfaces and Malignancy in Bethesda, the Conference on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasms in Bethesda, the Workshop on Persons at High Risk of Cancer in Key Biscayne, Florida, the Conference on Cancer Control and the Behavioral Sciences in San Antonio, Texas, the Third World Conference on Smoking and Health in New York City, and New Drug Seminars on Adriamycin and DTIC. Articles from these meetings appeared in news magazines for scientists and physicians as well as in newspapers and magazines for the public.

Articles on the National Cancer Program and the Cancer Control Program written by OCC staff appeared as part of an ACS-sponsored supplement on cancer in a Sunday issue of the Washington Post.

The staff handled approximately 800 inquiries from news reporters and issued 32 news releases and press summaries. Intense and continuing interest was generated by potential environmental and occupational causes of cancer, including asbestos, vinyl chloride, diethylstilbestrol (DES), alcoholic beverages, cyclamates, smoking, pesticides, chlorinated substances in drinking water, hair dyes, reserpine, ethylene dibromide, trichloroethylene, and aerosols as related to potential depletion of atmospheric ozone. Related inquiries concerned county-by-county statistics and maps illustrating U.S. cancer death rates, and the resolution of the National Cancer Advisory Board on smoking with the subsequent request by the President for scientific evidence supporting the resolution.

Breast cancer therapy, detection and rehabilitation received renewed attention when the Breast Cancer Task Force "Report to the Profession" coincided with breast cancer surgery for wives of the President and Vice President. Announcement of the isolation of a virus from cells of a patient with acute myelogenous leukemia generated national interest in virus research.

Members of the press also inquired about many other topics, including employability of cancer patients and long-term cancer survivors, costs of cancer care, cancer research funding and progress, Comprehensive Cancer Centers, Laetrile, the "Maruyama vaccine," and Dr. Albert Sabin's retraction of findings published in 1973 relating a type of herpesvirus to nine forms of cancer.

OCC responded in the Washington Post to an article criticizing the NCP, NCI and ACS. A more detailed response entitled "Cancer--Now The Good News" was prepared for the Director's signature and has been widely circulated, including insertion in The Congressional Record.

Research and cancer control activities of the National Cancer Program received increased coverage by foreign journalists. Visits to NCI for interviews and filming included reporters from Belgium, Canada, England, Italy, the U.S.S.R., and West Germany.

OCC staff assisted Science magazine science writers in preparing a series of articles on cancer research, including NCP research programs. The series was later published as a book.

Radio and Television

During the year the Institute cooperated with numerous radio and television stations to deliver information about topics including carcinogens in drinking water, breast cancer detection, occupational hazards, and the psychological aspects of cancer.

NCI spokesmen participated in programs including "Not for Women Only," "Face the Nation," "Mike Wallace Show," and broadcasts on National Public Radio, the Physician's Radio Network, Voice of America, and the Belgian and Canadian national television networks. NCI staff also worked with the Children's Television Network to include segments about cancer on "Feeling Good," a weekly health series for adults which premiered in November. The British Broadcasting Corporation and the "NBC Nightly News" interviewed NCI scientists for programs to be broadcast later in 1975. Local stations from Hollywood, California to Portland, Maine used the Office of Cancer Communications as a resource.

"Progress Against Cancer," a 28-minute film starring Danny Thomas, Marlo Thomas, John Wayne, Dionne Warwick and Anne Baxter in addition to prominent cancer scientists, was released for use by television stations nationwide. During this first year of distribution, it was shown on approximately 200 local stations to a total audience of almost five million.

Other Activities

Approximately 25 speeches, proclamations, and special messages of congratulation to individuals or organizations were drafted for use by the President; members of Congress; the Secretary, HEW; and the Director, Deputy and Assistant Directors and senior staff of the Institute.

The OCC prepared a number of articles and other documents for the Director's by-line, including "Research and the National Cancer Program" for *Science* magazine; "Cancer Program is Well Underway" for *U.S. Medicine*; a background document for the Postgraduate Course on Gastrointestinal Cancer, conducted by the American Gastroenterological Association; "New Bill Extends War on Cancer" for the *American College of Radiology Bulletin*; a foreword for a series of articles on Topics in Oncology for the *Journal of the American Medical Association*, and the Director's opening statement, to be read at the FY 1976 Congressional Appropriations hearings.

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1974 through June 30, 1975

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33912	Mount Sinai School of Med. Rubin, A. D.	Studies of the Biochemical Means by which Effector Molecules bind to Mammary Cell Surfaces & Influence Cellular Biochemistry	R-76
33909	Medical College of Georgia Scott, D. F.	Studies on the Role of 3', 5'-Cyclic Adenosine Monophosphate & Related Enzyme Systems in Mammary Gland Neoplasia	R-77
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33903	Goldwater Memorial Hospital, N.Y. U. Post, J.	Cell Kinetics of Breast Cancer	R-87
	The Milton S. Hershey Med. Ctr. Pennsylvania State U. Santen, R. J.	Suppression of Endocrine Function by Systemic Agents as Treatment of Human Breast Cancer	R-88
	Evanston Hospital Scanlon, E. F.	Therapy of Patients with Stage II or Stage III Carcinoma of the Breast	R-89
43899	Allegheny General Hospital Schiffer, L. M.	Cell Kinetics of Breast Cancer	R-90
43917	California Univ. Los Angeles Sparks, F. C.	Therapy of Patients with Stage II & Stage III Carcinoma of the Breast	R-91
43916	Emory U. School of Medicine Vogel, C.	Study of Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients	R-92

BCTF Treatment (cont'd)

43867	Worcester Found. for Exper. Biol. Welsch, F.	Estrophile Binding & Estrophile Proteins in Human Breast Cancer	R-93
33881	Scripps Clinic & Research Found. VanderLaan, W. P.	Prolactin Radioimmunoassays in Human Serum	R-94
33861	Papanicolaou Cancer Research Inst. Zucker, R. M.	New Techniques in Cell Kinetics of Breast Cancer	R-95

Cancer Diagnosis Contract Program

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Contract Research Summaries

General Diagnosis

Contract No. NO1-CB-	Institution Investigator	Title	
53865	Alabama Univ. Barnes, G. T.	Development & Testing of a System to Improve X-ray Imaging, Contrast and Sharpness by Elimination of Scatter, While Retaining the Resolution of Film	S-5
43974	American Coll. of Radiology Present, A. J.	Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy	S-6
43978	Baylor College of Medicine Hazlewood, C. F.	Nuclear Magnetic Resonance to be Studied in Neoplastic & Non-neoplastic Tissues	S-7
53863	Boston Univ. Albert, A.	Determination of Optimal Frequency of Screening Strategies	S-8
43976	Chicago Univ. Moossa, A. R.	Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage & Prior to the Presence of Metastasis	S-9
43868	Cincinnati U. Medical Center Buncher, C. R.	Statistical Center for Cooperative Lung Cancer Groups	S-10

General Diagnosis Contract Program (cont'd)

43992	Hahnemann Med. Coll. and Hospital Brady, L.W. & Stanton, L.	Clinical Application & Evaluation of "Xonics" Electron Radiography	S-11
43903	Harbor Gen. Hosp. Odell, W.	Hormone Markers for the Detection & Diagnosis of Cancer	S-12
45037	Johns Hopkins U. Frost, J. K.	Lung Cancer Control: Detection and Treatment	S-13
92172	Johns Hopkins U. Frost, J. K.	Lung Cancer - Early Detection, Localization and Therapy	S-14
43911	Johns Hopkins U. Hollis, D. P.	Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-neoplastic Tissues	S-15
43968	University of Louisville Foundation, Inc. Songster, C. L.	Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or More (over 7-10 mls per 24 hours) of Human Blood in Human Feces	S-16
53886	Mayo Foundation Fontana, R. S.	Detection and Localization of Early Lung Cancer	S-17
23854	Mayo Clinic Go, Vay Liang W.	Carcinoembryonic Antigen (CEA) Test, a New Method and Its Evaluation	S-18
43895	Mayo Foundation Go, Vay Liang W.	Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis	S-19
43975	Memorial Hosp. for Cancer & Allied Diseases Fitzgerald, P. J.	Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis	S-20
45007	Memorial Hosp. for Cancer and Allied Diseases Melamed, M. R.	Lung Cancer - Early Detection Localization and Therapy	S-21
53907	Michigan Cancer Foundation Brennan, M. J.	Determination of Optimal Frequency of Screening Strategies	S-22

General Diagnosis Contract Program (cont'd)

53906	Minnesota Univ. Gilbertsen, V. A.	Design of an Experiment to Assess the Impact of Multisite Screening on Total Cancer Mortality	S-23
53862	Minnesota Univ. Gilbertsen, V. A.	The Use of Screening Technique for Blood in the Stool as a Means of Detecting Early Cancer of the Bowel	S-24
43870	Mount Sinai School of Medicine Rosenfield, R. E.	Development of a Simple, Quick Accurate Method of the Detection of Small Amounts or More (over 7-10 mls per 24 hours) of Human Blood in Human Feces	S-25
33855	Institute of Environmental Medicine Lippmann, M.	Study of Intrabronchial Deposition of Inhaled Radioopaque Particles	S-26
43979	Downstate Med. Center, SUNY Damadian, R.	Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues	S-27
43977	SUNY at Buffalo Barnard, E. A.	Biochemical Markers or Enzyme Changes that may Presage the Presence of Cancer	S-28
43896	Rochester Univ. School of Med. and Dentistry	Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy	S-29
43902	Stanford Univ. School of Med. Lederberg, J.	Biochemical Markers or Enzyme Changes that may Presage the Presence of Cancer	S-30
53864	Tennessee Univ. Davies, D. F.	Design of an Experiment to Assess the Impact of Multisite Screening on Total Cancer Mortality	S-31
53914	Wisconsin Univ. Siedband, M. P.	Development & Testing of a System to Improve X-ray Imaging Contrast and Sharpness by Elimination of Scatter While Retaining the Resolution of Film	S-32

Cancer Diagnosis Radiology Contract Program

40302	ERDA-Lawrence Berkeley Lab. California U. Berkeley Tobias, C. A. & Benton, E. V.	Exploration of the Use of a Proton Beam in Tissue Densitometry	S-33
50304	ERDA - Lawrence Berkeley Lab California U. Budinger, T. F.	Algorithms for Computerized Transaxial Nuclide Reconstruction	S-34
43918	Chicago Univ. Skaggs, L. S.	Exploration of the Use of a Proton Beam in Tissue Densitometry	S-35
43910	Columbia Univ. Coll. of Physi. & Surgeons Hilal, S. K.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	S-36
43980	Cornell Univ. Med. College Potts, D. G.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	S-37
43981	George Washington University Davis, D. O.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	S-38
43983	Massachusetts Gen. Hospital New, P. F. J.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	S-39
53859	Massachusetts Gen. Hospital Correia, J. A.	Algorithms for Computerized Transaxial Nuclide Reconstruction	S-40
43982	Mayo Foundation Baker, H. L.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	S-41
53857	Mayo Foundation Robb, R. A.	Construction of a Projection Data Base for Testing Algorithms for Computerized Transaxial Reconstruction	S-42

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53913	Pennsylvania U. Kuhl, D. E.	Algorithms for Computerized Transaxial Nuclide Reconstruction	S-43
53858	Presbyterian Hosp. Columbia-Presbyterian Med. Ctr. Hilal, S. K.	Development and Evaluation of Computerized Transaxial X-ray Reconstruction	S-44
53860	Research Found. of SUNY Herman, G. T.	Algorithms for Computerized Transaxial X-ray Reconstruction	S-45
55097	Arizona Univ. Present, A. J.	Demonstration Project in Earlier Detection of Breast Cancer	S-46
35024	Cincinnati U. Moskowitz, M.	Demonstration Project in Earlier Detection of Breast Cancer	S-47
45064	Duke University Lester, R. G.	Demonstration Project in Earlier Detection of Breast Cancer	S-48
45058	Albert Einstein Med. Ctr. Isard, H. J. Temple U. Hosp. Lapayowker, M.	Demonstration Project in Earlier Detection of Breast Cancer	S-49
35016	Emory Univ. Brown, R. L. Egan, R. L. Georgia Baptist Hosp. Letton, A. H.	Demonstration Project in Earlier Detection of Breast Cancer	S-50
45095	Ellis Fischel State Cancer Hospital Donegan, W. L.	Demonstration Project in Earlier Detection of Breast Cancer	S-51
45062	Georgetown Univ. Abernathy, M.	Demonstration Project in Earlier Detection of Breast Cancer	S-52
45088	Good Samaritan Hosp. and Med. Ctr. Goodman, M. J.	Demonstration Project in Earlier Detection of Breast Cancer	S-53

Cancer Diagnosis Radiology Contract Program (cont'd)

35004	Guttman Breast Inst. Strax, P.	Demonstration Project in Earlier Detection of Breast Cancer	S-54
45067	Iowa Lutheran Hosp. Young, D. C.	Demonstration Project in Earlier Detection of Breast Cancer	S-55
35026	Kansas U. Med. Ctr. Humphrey, L. J.	Demonstration Project in Earlier Detection of Breast Cancer	S-56
35012	Louisville Univ. Moore, C.	Demonstration Project in Earlier Detection of Breast Cancer	S-57
35028	Virginia Mason Hospital Carlile, T.	Demonstration Project in Earlier Detection of Breast Cancer	S-58
45068	Samuel Merritt Hospital Schweitzer, R.	Demonstration Project in Earlier Detection of Breast Cancer	S-59
45049	Michigan Univ. Threatt, B.	Demonstration Project in Earlier Detection of Breast Cancer	S-60
35029	Mountain States Tumor Institute Ward, E.	Demonstration Project in Earlier Detection of Breast Cancer	S-61
35006	College of Med. & Dentistry of New Jersey Rush, B.	Demonstration Project in Earlier Detection of Breast Cancer	S-62
35020	Oklahoma Univ. Health Sci. Ctr. Haberman, J. D.	Demonstration Project in Earlier Detection of Breast Cancer	S-63
45046	Pacific Health Research Inst. Gilbert, F. I.	Demonstration Project in Earlier Detection of Breast Cancer	S-64
45065	Pittsburgh Univ. Fisher, B.	Demonstration Project in Earlier Detection of Breast Cancer	S-65

Cancer Diagnosis Radiology Contract Program (cont'd)

45096	Rhode Island Hosp. Constantine, H. P.	Demonstration Project in Earlier Detection of Breast Cancer	S-66
55100	St. Joseph's Hosp. Moore, D. L.	Demonstration Project in Earlier Detection of Breast Cancer	S-67
55210	St. Vincent's Med. Center McClow, M. V.	Demonstration Project in Earlier Detection of Breast Cancer	S-68
45098	University of Southern Calif. Guiss, L. W.	Demonstration Project in Earlier Detection of Breast Cancer	S-69
55099	Vanderbilt Univ. Hospital Burko, H.	Demonstration Project in Earlier Detection of Breast Cancer	S-70
45047	Wilmington Med. Center Whitney, L. W.	Demonstration Project in Earlier Detection of Breast Cancer	S-71
35018	Medical College of Wisconsin Youker, J. E.	Demonstration Project in Earlier Detection of Breast Cancer	S-72

Cytology Automation Contract Program

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Contract Research Summaries

Contract No. NO1-CB-	Institution Investigator	Title	
43960	Alabama University West, S.	Develop and Evaluate New Stains and other Optical Markers Useful for Gynecologic Cytopathology Specimens	T-3
43919	Chicago Univ. Bibbo, M.	Studies and Investigations of the Diagnostic Value of Gynecologic Cytopathology Samples	T-4

Cytology Automation Contract Program (cont'd)

43945	Karolinska Inst. Caspersson, T.	Develop and Evaluate New Approaches to the Problem of Markers applicable to Gynecologic Cytopathology Specimens	T-5
43984	Pennsylvania State University Todd, P.	Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens	T-6
43963	Montefiore Hosp. & Medical Center Koss, L. G.	Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens in Suspension	T-7
43955	SUNY - Downstate Medical Center Tolles, W. E.	Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathologic Specimens in Suspension and on Slides	T-8
43937	Temple U. Med. School & Hosp. Hontz, A. & Greenwald, E.	Vaginal and Cervical Cell Sample Sources for Cytology Automation	T-9
43943	Polysciences, Inc. Halpern, B. D.	Immunological, Enzymatic and other Biochemical Markers for Detection of Abnormal Cervical Cells on Slides or in Suspensions by Optical Methods	T-10
33873	Chicago Univ. Wied, G. L.	Evaluation of Information Requirements for Image Processing of Cell Samples as Basis for Development of Automated Cell Recognition System	T-11
40300	Lawrence Livermore Laboratory Mayall, B. H.	Development and Evaluation of New Stains and other Optical Markers Useful for Cytopathologic Specimens in Suspension	T-12

Cytology Automation Contract Program (cont'd)

43962	Papanicolaou Cancer Research Institute Leif, R. C.	Develop and Evaluate New Stains and other Optical Markers Useful for Clinical Cytopathological Specimens in Suspension	T-13
43912	Louisiana State U. Lillie, R. D.	Development of Hematoxylin Substitutes for Diagnostic Staining of Tissues and Exfoliated Cells	T-14
Y01-CB-10055	AEC-Los Alamos Scientific Lab. Steinkamp, J. A.	Application of High-Speed Flow System Methods of Cell Analysis and Sorting to Cancer-Related Cell Populations	T-15
33862	Rochester Univ. Wheeless, L. L.	Investigation of a Slit-Scan Technique as a Basis for an Automated Prescreening System for Cancer Detection in Cytology	T-16
New	SUNY - Downstate Medical Center Tolles, W. E.	Vaginal and Cervical Cell Sample Sources for Cytology Automation	T-17
New	American Science & Engineering, Inc. Cobb, C.	A Pre-clinical Evaluation of ^{67}Ga as a Marker for the Automated Detection of Neoplasia of the Uterine Cervix	T-18
New	Nuclear Leasing Corporation Ehrlich, M. P.	Supply Cytoscreener and Technician	T-19
New	Rochester Univ. Wheeless, L. L.	Investigation of High Resolution Fluorescence Measurements on Cells from the Human Female Genital Tract & Quantitative Study of Acridine Orange and other Fluorochromes	T-20
New	Utah Biomed. Test Laboratory Grover, T. P.	Evaluation of Cytoscreener	T-21
New	D.C. General Hosp. Bepko, F. J.	Vaginal and Cervical Cell Sample Sources for Cytology Automation	T-22

SUMMARY REPORT
OF THE
DIRECTOR
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE

July 1, 1974, Through June 30, 1975

INTRODUCTION

The Division of Cancer Biology and Diagnosis was established in August, 1972, to enhance the research program in the two major directions - diagnosis and biology. In addition, the Division supports the Breast Cancer Task Force Program, which expands to a limited extent into organ site oriented research in epidemiology and treatment. A close coordination of efforts is maintained with the Cancer Control Program in areas of overlapping interest.

The Division's research program may be divided into three broad areas: cancer biology, immunology, and clinical research. The organizational structure roughly follows this same division: first, a group of laboratories performing basic research in cancer biology immediately under the Director; second, three elements, the Immunology and Immunodiagnosis Branches and the Laboratory of Cell Biology, working in the immunobiology, immunodiagnosis and immunotherapy of cancer, assigned together with a sizeable contract program to an Associate Director for Immunology; and third, clinical branches working intramurally in the fields of surgery, radiation, dermatology, metabolism, and pathology. Clinical research includes both cancer diagnosis and treatment. A fourth organizational element, the office of the Associate Director for Program Planning, performs certain broad administrative functions, and also includes three major contract programs: Cancer Diagnosis, Cytology Automation, and Breast Cancer Task Force. There is considerable crossover of research directions between the various research laboratories and clinical branches, and also between major program divisions.

Among the organizational highlights experienced last year, noteworthy is the departure of two program leaders: Dr. N. I. Berlin, Director, left for Northwestern University, and Dr. A. Ketcham took a job at the University of Miami. The new Director - Dr. A. Rabson - took over in the second half of this fiscal year. The position of the Associate Director for Clinical Research was transferred to the Division of Cancer Treatment, as were two major treatment branches: Surgery and Radiation Oncology, in a move to coordinate all treatment effort under a single leadership. This report, however, will include above branches, since the transfer occurred late in the reporting period. Two new units were added to the Divisional strength: a Laboratory of Immunodiagnosis was created and the Image Processing Unit was transferred to the Diagnosis Branch from the Division of Cancer Treatment.

The Laboratory of Biochemistry completed its first year in new organizational form.

The work in the Biosynthesis area has been carried out in four directions:

1. Continuation of the study of mouse myeloma DNA polymerizing enzymes. DNA polymerase I was resolved into two forms and characterized with respect to physical and enzymatic properties. It was found that when freshly isolated human cells were fused with mouse cells and grown in culture, the human genes could alter the amounts of murine type-C particle components present in the cell or released into the medium. Regulation of packaging and release of C-particle DNA polymerase may be attributed to genes on four human chromosomes.

2. The study of basic mechanisms of collagen biosynthesis and secretion and the factors that regulate these processes in cultures of normal and transformed fibroblasts have continued. Underhydroxylated collagen protein secreted into tissue culture medium is much smaller than procollagen, a result that suggests underhydroxylated collagen is susceptible to enzymatic degradation in cell cultures. Further support has been gained for the conclusion that transformed 3T3 cells synthesize a reducing agent that functions in the prolyl hydroxylase reaction. Finally, it was found that di-butyryl cyclic AMP (dbc AMP) did not affect the levels of pryolyl hydroxylase activity under conditions in which the relative rate of collagen synthesis was increased and that an observed increase in proline transport caused by dbc AMP was probably not related to cyclic AMP metabolism but to butyric acid.

3. The study of mouse tumor-associated type-A intracysternal particles showed that three main structural proteins of A-particles contain repetitive sequences and the amino terminal residue of all three proteins is argenine. The repetitive sequences appear to be connected linearly through peptide bonds. In an attempt to examine the A-particle genome, a poly(rA)-containing RNA was isolated from purified A-particles. A study of the expression of A-particles and C-particles induced by treatment of N-4 cells with IUdR and dexamethasone showed elevation of C-particle markers, but no increase in A-particle markers.

4. The study of the structure of macromolecules showed that the shape of vesicular stomatitis virus is similar to that of plant rhabdoviruses. It has been proposed that phosphoribosylpyrophosphate synthetase is made up of five subunits in asymmetrical quarternary arrangement. In a second project, it was found that in culture, plasmablasts and mature plasma cells formed very prominent, long-term associations with macrophages. Comparison by scanning electron microscopy of the surface morphology and cell interactions between lymphocytes and macrophages in vivo and in vitro showed interactions to be present in both environments, but the surface of the macrophage was profoundly altered by contact of the cell with the foreign substrates, Millipore filter and glass.

Studies on rat hepatoma x mouse fibroblast hybrids indicated that glucocorticoid receptors are functionally different in the two cell types. This suggests an active role for physically similar but subtly different receptors for steroids that induce cell-specific responses. In somatic cell hybrids of the same type, the two non-contact-inhibited parents combined to give contact-inhibited hybrid progeny. Contact-inhibited behavior correlated with a full complement of chromosomes from both parents. When injected into "nude" mice, the contact-inhibited cells showed a marked delay in tumor production, and the recovered tumor cells had a reduced chromosome number.

Sheets of human epidermal cells derived from skin and gingiva have been successfully cultivated. With these cultures the metabolism of benzo(a)pyrene has been demonstrated and an unusual spectrum of quinones, epoxides and arylhydroxides has been isolated, identified and quantitated. It is believed that in order for carcinogenesis to occur, the procarcinogen must be metabolized to an active intermediate that results in effecting the neoplastic transformation.

Studies on the susceptibility of neoplastic cells to BCG-activated macrophages showed that the degree of susceptibility of established cell lines correlates with their tumorigenicity; BCG-activated macrophages can be used as an *in vitro* assay. Other *in vitro* assays of neoplasia, such as selective growth in agarose and the 24-hour colony morphology also correlate with tumor development *in vivo*.

In attempts to control the karyotypic instability of mouse cells in culture that may be a causative factor in "spontaneous" neoplastic transformation, previous observations that fetal bovine serum (FBS), as compared with horse serum (HS), promotes karyotypic stability in freshly isolated mouse cells has been confirmed. Six morphologic features of cultured cells have been found to show high coefficients of correlation with the neoplastic state, suggesting a relationship. A method for measuring one of these, parallel orientation, is being evaluated for computer programming. Three of the other morphologic features are being quantitated. The objective of this study is to provide quantitative methods for cytodiagnosis of the neoplastic state in cultured cells.

Cell surface features have been considered indices of neoplastic transformation. Drastic changes in surface morphology reportedly occur as cells progress through the mitotic cycle. Cell surfaces have been examined during the mitotic cycle by scanning electron microscopy and cinephotomicrography in a clone of normal and derivative neoplastic cells. The conclusion drawn from this study is that cell surface morphology does not change predictably as these cells progress through the mitotic cycle and is not significantly altered in neoplastic transformation.

The work on histidine biosynthesis included isolation of mutants of a new class, in which mutations in the gene that specifies the first enzyme for histidine biosynthesis cause constitutive expression of the histidine operon, and studies that show that the first enzyme for histidine biosynthesis binds specifically to the DNA of the regulatory region of the histidine operon *in vitro*. These studies have provided strong support for previous conclusion that the first enzyme for histidine biosynthesis plays a direct role in autogenously regulating expression of the histidine operon.

A fast purification procedure for polynucleotide phosphorylase has been developed. The method is based on affinity chromatography with poly(U)-Sepharose, allowing high yields of homogeneous, primer-independent enzyme. Structural and subunit studies on this enzyme, as well as the primer-dependent enzyme generated from it by limited proteolysis, are proceeding. A new assay procedure for cyclic-AMP phosphodiesterase was developed. The method does not require the conversion of the product AMP to adenosine. The assay is being used for the purification of particulate and soluble phosphodiesterase. The mechanism of action of the enzyme and its regulation by interaction with activators and inhibitors will be investigated.

The studies on the transfer of genetic information by incubation of recipient cells with chromosomes identified as human in origin the hypoxanthine phosphoribosyltransferase gene products of four independent clones obtained by transforming mouse Aq cells with human metaphase chromosomes. However, human species of two other X-linked enzymes, glucose-6-phosphate dehydrogenase and phosphoglycerate kinase, were not found in any of these clones. Thus, only a portion of the X chromosome was expressed after transfer.

Of 11 clones obtained by selecting for the presence of thymidine kinase (TK) after transformation of TK-deficient B_{82} mouse cells with Chinese hamster chromosomes, two also contained the chromosomal donor species of galactokinase, indicating that closely linked genes are sometimes transferred together in this procedure. The clones were examined for the chromosomal donor species of nine nonlinked enzymes, but none were detected, establishing that these clones had not arisen through cell fusion.

In another study it was discovered that when antigen-reactive spleen cells are incubated on and briefly centrifuged on allogeneic target cell monolayers, some of the cells bearing graft vs. host activity are bound specifically. An attempt is now being made to ascertain which specific determinants must be present on the target cell monolayers for adsorption to occur.

The work of the Laboratory of Biology continues to be focused on mammary tumorigenesis and related subjects. Much of the work is carried out on defined genetic material in characterized inbred strains of mice with more recent attempts to transfer the problems to cell culture of these tissues and of tissues from human sources. One approach is through biochemistry and biochemical genetics describing genetic control of biochemical differences between the inbred strains, defining and measuring enzymatic and hormonal differences between normal, developing and neoplastic mammary tissue and between viral infected and noninfected tissues, and determining the gene regulation of these differences to gain more insight into the mechanisms of tumorigenesis.

The work on catalase received considerable emphasis. Specific genetic loci control the activity of this enzyme in specific organs of the mouse. Liver catalase is controlled by the Ce-1 gene as yet not located in the genome. Erythrocyte catalase is controlled by the Cs-1 gene now shown to be on chromosome 2 approximately 26 centi-Morgans from the agouti locus. Isozymes of kidney catalase have been shown to be controlled by a single autosomal locus with two alleles apparently linked with the H-2 complex on chromosome 17.

An attempt is being made to ascertain whether or not the mammary tumor virus information in the mouse is transmitted as a single dominant gene as has been hypothesized. Segregation ratios obtained indicate that there is a major dominant gene controlling antigen expression but this is not the only gene involved. This major dominant gene is not the viral information itself, but is a controller gene regulating the expression of the viral information presumably located elsewhere in the host genome, possibly not as a specific locus and possibly occurring in all mice.

A major finding has been a high molecular DNA unique to the intracytoplasmic A particle that has been thought to give rise to the B particle, the mature MTV, a RNA virus. The presence of this DNA in the A particles was confirmed by incorporation of ^{3}H-thymidine (a DNA tracer) in tumor tissue with A particles and observing association of grains with the A particles in the autoradiographs. This A particle DNA has been characterized. RNA was also found in the purified A particles, but it was of low molecular weight in contrast with that in M MTV. Earlier SDS-polyacrylamide analyses had shown considerable disparity in molecular sizes of polypeptides of the A and B particles. A study now has been completed showing this disparity to be due to the presence of uncleaved M MTV structural proteins in the A particles.

Research in the Laboratory of Molecular Biology has been expanded into four areas of interest:

1. The role of cyclic AMP in malignant transformation.
2. Gene activity control and nucleotide sequences at regions where transcription begins and terminates.
3. Mechanism of genetic recombination.
4. Formation, storage and release of thyroid cell and factors controlling its growth, differentiation and conversion to a malignant cell.

Transformed embryonic fibroblasts have low levels of cyclic AMP and their decreased adhesiveness, rounded shape, rapid growth and increased agglutinability of transformed cells are due to low levels of cyclic AMP. To understand why cyclic AMP levels are low in transformed cells, detail characterization of the enzyme that synthesizes cyclic AMP, adenylate cyclase and the enzymes of cyclic AMP degradation, the cyclic AMP phosphodiesterases, was undertaken. In transformed NRK cells and CEF, the activity of adenylate cyclase is diminished, and this low activity appears to account for low cAMP levels. In some cases, cAMP phosphodiesterase activity is also low. Decreased cAMP phosphodiesterase is secondary to low cAMP levels; cAMP is an inducer of cAMP phosphodiesterase.

Ultrastructural studies on the effects of Bt$_2$cAMP on cell shape showed that cAMP induces the formation of thick bundles of microfilaments particularly at cellular margins and these are areas where the cell is closely opposed to the substratum. Using dark-field microscopy, it was noted that transformed cells have microvilli which rapidly disappear upon addition of Bt_2cAMP. The reason that transformed cells are agglutinated by plant lectins is because microvilli interdigitate and hold cells together. cAMP treatment lowers agglutinability by causing resorption of microvilli.

In order to understand how the cAMP-CRP complex enables RNA polymerase to initiate gene transcription, the effect of denaturing conditions, high temperature, low Mg^{++} or low salt, on the formation of a DNA:RNA polymerase pre-initiation complex, was measured, indicating that cAMP-CRP acts by locally denaturing DNA at promoter loci. Another nucleotide that stimulates transcription is ppGpp (guanosine tetraphosphate). A protein which is required for ppGpp to act was found and purified.

The product of gamma gene (N) causes RNA polymerase to fail to obey normal transcriptional stop signals, resulting in a very large and apparently stable species of RNA. It appears as if the N gene product antagonizes the action of termination factor rho. In light of this, the nucleic acid structure involved in the control of transcriptional termination has been determined and new methods for sequence analysis developed. The 3'-terminal sequences of a number of transcripts from gamma and gamma gal templates were examined, showing that all of the transcripts examined have 3'-terminal sequence heterogeneity consisting in the addition of 1-5 adenylate residues, and that the termination factor rho results in a structural change in the termination sequence.

In the area of thyroid cell function, it has been established that: The location of newly formed iodoprotein (30 sec after ^{125}I injection) is in the thyroid lumen, indicating iodination is an extracellular event; $^{45}Ca^{++}$ is first taken up by the thyroid cell and later concentrated in the lumen where it binds to thyroglobulin and changes its physical properties. The thyroid epithelial cell can be induced to phagocytize red cells and debris from the thyroid lumen by a process different from macropinocytosis and apparently independent of TSH. Mitosis of the thyroid cell can occur without the cell losing contact with adjacent cells in the follicle or the lumina or basal lamina.

The research in the Laboratory of Physiology continued in the areas of metabolism of the regulation, energy expenditures and the interdependence of metabolic and behavioral responses, control of the synthesis of proteins and nucleii acids, molecular and biological effects of radiation, and search for agents with antitumor activity.

Change in diurnal variation of feeding and motor activity in cachectic tumor-bearers is similar to change in diurnal variation induced by chronic hypothalamic damage. This is the first evidence of a connection between the cachectic process and dysfunction of central control of feeding. Feeding responses to nutrient density, environmental temperature and exogenous insulin are distorted by tumor growth. In general, feeding responses of tumor-bearers to feeding stimuli are attenuated, but there appears to be substantial dependence on the effectiveness of the precancerous control of feeding of the host.

In mammalian tissues, uridine-cytidine kinase is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction is rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end products. This enzyme was purified 1800-fold from the mouse ascites mast cell neoplasm P815/S and characterized with respect to stability, pH optimum, divalent cation requirement, and substrate specificity. The enzyme exhibited a broad specificity for the phosphate donor substrate, but a lesser one for the phosphate acceptor substrate. The ratio of uridine to cytidine kinase activity remained constant throughout the purification of the enzyme. The purified enzyme has a molecular weight of at least 150,000.

The UMP-CMP kinase has been purified 300-fold from Tetrahymena pyriformis, a rapidly growing protozoan unable to synthesize the pyrimidine ring de novo and hence dependent on anabolism from the preformed ring. The purified Tetrahymena enzyme also retains capacity to phosphorylate dCMP. This is the first extensive purification for CMP-UMP kinase activity; this enzyme has now been renumbered as EC 2.7.4.14, ATP: (d)CMP phosphotransferase. This purified enzyme was most stable in phosphate buffer, had a pH optimum between 7.0 and 8.0, required Mg^{2+}, was inhibited by CDP, UDP, ADP, and high levels of ATP.Mg. Subsequent studies revealed that the mechanism of the enzymatic reaction was probably sequential by addition of both substrates to the enzyme to form a ternary complex. Although the reaction is freely reversible, physiologically the enzyme is intended to function primarily in the forward or anabolic direction. The sequential addition of CMP and $ATP.Mg^{2-}$ to the enzyme occurs in a random fashion.

Studies on immunogenetic factors influencing bone marrow transplantation in mice have continued with the goal of avoiding GVH reaction, so that this procedure can become an adjunct to heavy radiation or chemotherapy in the treatment of cancer. Marrow from the mother produces a less severe reaction in radiated mouse progeny than that from the father, presumably because of the persistence of tolerance for antigenic differences between mother and fetus. It was also noted that radiated daughter mice were more tolerant of mothers' marrow than radiated sons; this was true both for inbred and hybrid strains, suggesting that it is due to antigens controlled by the Y chromosome. These phenomena have now been studied by transplantation of fertilized ova of one strain into foster mothers of another. The results generally confirm the idea that phenotypic expression of the antigens of the fetus, and responsiveness as well, is reduced by maternal influences.

Radioprotective and radiosensitizing agents are being studied in order to develop new approaches to increase the differential radiation sensitivity of tumors. In the chain of events that lead to loss of biological activity, free radicals play an important role. Recently, it was found that addition of certain metal ions to DNA at a 1:100 ratio of metal ions:nucleotides can reduce the radical yield by a factor of 10. From the ESR studies of cupric-DNA complexes, it was inferred that cupric ions compete with the bases in DNA for electrons produced by X-radiation. Study of the effect of visible light on DNA in solid state, previously irradiated in the dark at 77°K showed a 75% drop of the ESR signal, suggesting that ionic radicals produced by X-radiolysis at 77°K in DNA and its constituents are light sensitive.

Earlier experiments have shown that some of the β-hydroxy-amino acids exhibited growth-inhibitory activity in microbial systems selected for antitumor screening and probable tumor-inhibitory action in some mammalian systems. Four isomers of N-chloroacetyl-β-hydroxynorleucine have been prepared in quantities permitting minimal testing in three mammalian systems. Microbial screening tests for antitumor action showed that non-acylated compounds were not growth-inhibitory. On the other hand, N-chloroacetylated derivatives show modest but significant antitumor activity. In light of this, N-acyl derivatives of other amino acids were prepared: Chloroacetyl-DL-allylglycine, chloroacetyl-DL-β-2-thienylalanine and chloroacetyl-DL-p-fluorophenylalanine.

The primary objective of the Laboratory of Pathophysiology is the understanding of conditions which determine tumor regression or initiation, regulation and arrest of growth of neoplastic tissues. One group of projects deals with the mechanism of mammary tumor regression secondary to hormonal deprivation or cyclic AMP treatment of the host. Previous work showed that a high level of lysosomal activity is not the primary cause of tumor destruction, as it occurs after hormone withdrawal, but a secondary event or "cleaning up" type of operation. This was based on the observations: (1) increase of lysosomal enzyme activity in some regressing tumors occurs when regression is well advanced; (2) arrest or severe impairment of protein and RNA synthesis does not alter the time sequence of regression; and (3) the lysosomal enzymes remain localized within the cells and do not spill over into the interstitial compartment.

Studying the role of cyclic AMP, it was found that: injections of dibutyryl cAMP may produce regression in some rat tumors, that regression does not depend on the level of cAMP in the tissue, but on the presence of the protein-binding capacity of the tumor cytosol, and that prostaglandin E_1 treatment of tumor slices, from both responsive and unresponsive lines, produced a significant induction of basal and NaF-stimulated adenyl cyclase and cAMP-phosphodiesterase only in the line regressing under dibutyryl cAMP treatment. Since the cAMP binding protein is related to the regulatory subunit of protein kinase, the activation of protein kinase in the two lines of tumors, regressing and growing after dibutyryl cAMP treatment, is studied. cAMP dependent protein kinase activity is increased by prostaglandin E_1 treatment only in tumors regressing after cAMP treatment, not in the cAMP unresponsive tumors. Moreover, the catalytic subunit of protein kinase is similar in both types of tumors, but the regulatory subunit appears to be different, and this difference is related to cAMP-responsiveness.

Other projects are concerned with events occurring in cells at rest or at the initiation of mitosis. Study of RNA in lymphocytes, resting or dividing, under stimulation by mitogens showed that the largest molecules of heterogeneous RNA are poorly represented in the pool of poly(A)-bearing heterogeneous RNA and maintain a rather constant average size during the processing period. Resting lymphocytes contain about 70% of their cytoplasmic ribosomes as single 80S monomers, more than 80% of which were found to be easily dissociable in high salt concentration. New rRNA enter native 40S and 60S subunits and polysomes; as the particles age, they accumulate in the pool of inactive free ribosomes. Following growth stimulation with phytohemagglutinin, protein synthesis increase reaching a peak at 14-16 hours. Two classes of poly(A)-bearing RNA were found in resting lymphocytes. In lymphocytes dividing after mitogen stimulation, the half life of mRNA deviates markedly from that found in resting lymphocytes. These observations are compatible with the following interpretation: The resting lymphocyte polyadenylates new heterogeneous RNA which survives longer. In resting lymphocytes, protein synthesis is minimal, and most ribosomes are inactive until stimulation with a mitogen occurs. Rapid activation of ribosomes is probably due to the availability of a "dissociation" factor, while mRNA appears to be limiting at maximum levels of protein synthesis.

Analysis of the sequence of events involved in the initiation of mitosis showed that poly(ADP-Ribose) is being synthesized at the end of S-phase and beginning of G2 phase. Substances such as adriamycin, which arrest cell cycle progression, also produce accumulation of cells with elevated poly(ADP-Ribose) polymerase activity, but this does not occur with substances such as bichloronitrosourea, which act on the late G2 phase. By the use of 5-methylnicotinamide, which inhibits poly(ADP-Ribose) synthesis, a variant of HeLa cells has been developed which continues to produce poly(ADP-Ribose) and also survives. The resistance to 5-Methylnicotinamide of poly(ADP-Ribose) polymerase appears to be higher in the resistant cell population.

The remaining studies are in the area of general tumor pathophysiology: temperature regulation by the solid tumor, expansion of the possibilities of the capillary cell culture unit, using induction of neovascularization in the rabbit's eye as an identification of neoplastic transformation of human mammary gland at an early stage. The procedure was proven effective in our laboratory for mouse mammary gland by Dr. M. Gimbrone.

The activities of the Laboratory of Theoretical Biology fall into several areas: the biophysics of membranes, developmental biology, molecular configurations, kinetic metabolic systems and mathematical and computational methodology and theory related to modeling.

Studies have been carried out on conductance changes produced by "Excitability Inducing Material" which provide insights into the gating of ion conducting pores. Ionophoric properties such as voltage dependent conductance, conductance fluctuations, ion selectivity and pH dependence were investigated for a number of substances: antibiotic released from A. Cloacae, peptide from eel electroplax membranes, dopamine-β-hydroxylase, hemocyanin, valinomycin, gramicidin. Since some substances act both as ionophores and as B cell mitogens, a relationship between the two is suspected.

Another problem related to membranes is the modeling of the mechanism related to adenylate cyclase activity in membrane preparation. A previous model has been refined to include two activated and one inactivated state of the enzyme, and a catalytic site that interacts with the substrate (ATP) and an inhibitor (HATP). This model has been tested and found compatible with the data, and equilibrium constants for these states have been determined.

Originally developed for liver cell membranes, the model is also being applied to interpret adrenal cell cyclase activity.

Analysis of mechanisms underlying determinations and transdeterminations in Drosophila is an experimental effort to verify a theory on control circuits in developmental biology. According to theory, the determination of wings, legs, antennas, etc., from imaginal discs in Drosophila are controlled by a circuit composed of at least four bistable states. A transdetermination is a chance switch of one or more circuits from one state to another. Efforts are under way to fuse cells from different imaginal discs to determine the dominance of the various circuits.

Several metabolic systems are being actively modeled, reaching a high level of complexity and sophistication and on-hand experience in dealing with large systems, and providing new insights into the theory and methodology of modeling as related to large systems. This activity serves as a pilot project in establishing model-systems for several important metabolic systems in man. The model previously developed for apo-B proteins using an endogenous label has been extended to exogenously labeled apo-B and apo-C protein studies. A more complete and extensive model has evolved now that covers apo-B and apo-C kinetics in very low density, intermediate density, low density and high density lipoproteins. These studies have now been complemented by endogenously labeled lipoproteins kinetics involving simultaneous FFA and leucine labeling of the triglyceride and apoprotein moieties. Kinetic studies of HDL apo-A lipoproteins have also been carried out and integration of this model with one previously obtained for HDL apo-C is in progress.

The modeling of the glucose-insulin system involves two phases. The insulin secretion modeling and the study of the control mechanism of insulin on glucose utilization is continuing. A refined model that permits a better understanding of peripheral (muscle) glucose utilization and that predicts the relations between arterial, venous and "hot hand" response curves for glucose has been worked out. Analysis is also in progress of insulin, proinsulin and c-peptide kinetic curves in the rat, in the hope of getting a better insight into the secretion and degradation mechanisms of insulin. Detailed modeling is also being carried out on the interconversions of glucose, lactate, alanine and glycerol, both in man and dogs. Some theoretical considerations related to these, involving the Krebs cycle, are also being formalized.

In connection with thyroid hormone and iodine metabolism, a model has been formulated to account for the kinetics of iodide (I^-), thyroxine (T_4) and triiodothyronine (T_3) in the mother sheep and its fetus. The model suggests that there is no T_3 utilization in the fetus and that T_3 transport across the placenta greatly favors the mother. The amniotic fluid emerges as an important store of both T_3 and T_4 hormones. A high T_3 gradient between fetal plasma and amniotic fluid was predicted by the model and subsequently confirmed experimentally.

IMMUNOLOGY

Tumor immunology research comprises the greatest part of the program of four laboratories in the Division of Cancer Biology and Diagnosis. Three of these, the Immunology and Immunodiagnosis Branches and the Laboratory of Cell Biology, are organized under the Associate Director for Immunology. Although the Metabolism Branch is not organizationally connected with this program, its research is summarized in this section. Tumor Immunology Contract Program is reported in the Contract section.

The Immunology Branch carries out basic immunobiology investigations with particular emphasis on the structure and function of cell membranes, cellular immunity, genetics of the immune response and transplantation. The information obtained is then applied to investigations of the interactions between the immune system and neoplastic cells, and to the diagnosis and therapy of malignant disease in animal models and in man. The function of cells, including cells of the immune system, is partially initiated and modulated by interactions between cell surface receptors and a variety of ligands. One such receptor on the B cell subpopulation of human lymphocytes is a receptor for antigen-antibody complexes or for aggregated IgG. This receptor has been partially characterized and shown to be distinct from surface Ig and the C3 receptor. It is specific for the Fc portion of complexed Ig. The Fc receptor on B cells is necessary for antibody dependent cell-mediated cytolysis. The Fc receptors of murine B lymphocytes are either identical to or closely associated with Ia cell surface antigens. T lymphocytes also appear to have Fc receptors but in lower numbers or with lower affinity for immune complexes. The Fc receptor has been used to develop a preparative technique for separating human lymphocytes into T and B cell populations. Attempts are being made to isolate and characterize the Fc receptor.

Studies are being performed in an attempt to understand the mechanisms by which lymphocytes kill target cells, using lipid bilayer membranes as models for the target cell membranes. When these model membranes contain DNP groups, and antibodies for DNP are allowed to interact with the membrane, subsequent addition of non-immune human lymphocytes causes an increased conductance of several orders of magnitude across the antibody coated lipid bilayers, suggesting that an increase in ionic permeability may be the basic mechanism responsible for target cell death. Previous studies in the antibody dependent cell-mediated killing system have shown that attacking cells undergo a marked shape change when they interact with target cells. Lymphocytes that interact with antibody coated plastic surfaces undergo identical rapid and dramatic morphologic changes. These changes, and the capacity of these cells to kill in the antibody mediated system, can be blocked by a variety of drugs. These studies suggest that the morphologic changes are a necessary part of the lymphocyte killing process and permit this process to be divided into a phase of morphologic change, which is then followed by the actual killing phase.

New antigens are being detected on human B lymphoid cells. In one system, sera obtained from multiparous members of the inbred religious sect, the Amish, have been shown to detect antigens expressed on B cells. These antigens have been shown to be serologically and chemically different from HL-A alloantigens. In a different approach to this problem, pretreatment of human lymphocytes with appropriately absorbed anti-HL-A antisera inhibits the binding of Ig complexes to the Fc receptors of B lymphocytes. In the mouse system, pretreatment of B cells with serum containing anti-Ia similarly inhibits binding of Ig complexes to Fc receptors. It is presumed that both the sera obtained from multiparous Amish women and the specifically chosen HL-A antisera contain antibodies with specificity for human cell surface Ia antigens.

In another study, peripheral blood lymphocytes from normal humans were found to consist of four subpopulations. The use of cell surface markers has permitted the separation of patients with variable immunodeficiency and panhypogammaglobulinemia into distinct groups that appear to differ as regards the nature of their fundamental defect. Patients with chronic lymphocytic leukemia could be separated into T and B cell types.

Treatment of cell surfaces with the enzyme neuraminidase alters them in such a way that they react with a "natural" antibody present in normal human serum and in some mouse serums. The distribution of antibody levels in patients with newly diagnosed malignancies and in patients with various forms of immunodeficiency did not differ from that of age matched control normals. Studies were also performed to determine the fate of labelled neuraminidase treated autologous lymphocytes in mice known to contain high levels of antibody reactive with neuraminidase treated cells. No important differences in the *in vivo* fate was detected.

Separation of subpopulations of normal mouse spleen cells using nylon column passage has revealed synergy between what are presumed to be T and B cells in the generation of cell-mediated cytotoxicity. Functionally different subpopulations of mouse T lymphocytes have been identified. Primary *in vitro* sensitization of spleen cells against NIP-modified syngeneic cells generates cytotoxic cells specific for cells modified with the hapten NIP. *In vivo* sensitization with NIP-modified syngeneic cells generates memory cells but no cytotoxic cells, suggesting that cytotoxic T cells are a different population from the T cells that express memory for cytotoxic response.

In an attempt to define the defect in the lymphoid system of AKR mice which predisposes to the high spontaneous incidence of thymic lymphoma and lymphocytic leukemia, it has been shown that spleens and lymph nodes of leukemic animals contain an increased proportion of theta positive cells, and there is a disproportionately greater loss of con A responsiveness than of PHA in preleukemic and leukemic AKR mice. This leads to a progressively greater PHA to con A stimulation ratio as animals age and become leukemic. These results suggest that there is a con A responsive subpopulation of cells and that this population is more readily inactivated by murine leukemia virus.

Sera fractionated by gel filtration and ion exchange chromatography produce results indicating that cytotoxic antibody can be separated from lymphocyte dependent antibody. It is not known whether these represent functional differences amongst subclasses of IgG or some other factor. Human T cells are separated from B cells by forming rosettes and separating cell populations in a gradient. These studies suggest that cells with B characteristics were primarily responsible for target cell destruction. A different technique for separation shows that nonadherent cells (primarily T cells) are severely depleted of killing activity in the antibody dependent killing reaction.

The demonstration of genetic control of immune responses has opened an important pathway in gaining an understanding of the biology of the immune system. In studying genetic control of immune responses, it is desirable to utilize antigens of known structure. Staphylococcal nuclease meets this requirement. H-2 linked genetic control of antibody synthesis has been demonstrated and localized to the Ir I-B region. Cell-mediated immunity to this antigen measured in vitro was also shown to be under genetic control. The genetic control of cell-mediated immunity to synthetic polypeptides and to staphylococcal nuclease was also determined with an in vivo assay. It was clearly demonstrated that cell-mediated immunity is under genetic control and that the genes concerned are H-2 linked Ir genes. The in vivo delayed hypersensitivity was transferable by T cells but not by B cells from the immunized animals. Non-H-2 linked antibody responses have been demonstrated for lysozyme and synthetic polypeptides. The ability of irradiated mice to reject hamster bone marrow transplants is also under non-H-2 linked genetic control.

Hemopoietic histocompatibility antigens are phenotypically expressed only on blood forming and leukemic cells; they are capable of stimulating host versus graft reactions which are radioresistant in vivo. An in vitro system was developed in which F1 responding lymphocytes generate cytotoxic effector cells against spleen cells of H-2^b parents but not against H-2^a parents. This should make it possible to experimentally explore this complex system of antigens which are regulated by multiple H-2 linked genes.

Cell-mediated immunity can be induced in vitro against hapten modified target cells. It has been shown that the antigen developed on the cell surface consists of a hapten modified protein which is itself an H-2 antigen under the genetic control of either the D or K end of the H-2 complex. The immune response is not directed against the hapten itself, but rather against the hapten-modified cell surface proteins. The capacity to develop an immune response to the hapten-modified cell surface protein is also under genetic control which is H-2 linked and which maps to the left of the Ia genetic region.

Studies are being carried out to determine whether Ir genes control the immunoglobulin variable regions that are produced in "genetically controlled" antibody responses. Specifically purified mouse antistaphylococcal nuclease antibodies have been prepared and used to immunize rats. Rats have made antibodies against the mouse antibodies and by several criteria these antisera have been shown to contain antibodies specifically directed against idiotypic determinants. These rat antibodies have the capacity to react with the combining site of the mouse antistaphylococcal nuclease antibodies as evidenced by their ability to inhibit the capacity of the mouse antibody to inactivate staphylococcal nuclease in an enzymatic assay.

Ia antigens have been shown to be preferentially expressed on B cells and appear to be associated with or responsible for the stimulation of mixed lymphocyte culture reactions. The use of more sensitive techniques, including the fluorescent activated cell sorter, have demonstrated that Ia antigens are also present on at least some T cells. Anti-Ia antibodies have been found to specifically block the Fc receptor of B lymphocytes. This has led to the hypothesis that Ia antigens and the Fc receptor are either identical cell surface components or that they are physically located so closely together that immunologic reactions at the site of one of these components sterically blocks the functional reactivity of the other. The structure of Ia antigens has been studied using specific immune precipitation followed by analysis on polyacrylamide gels. Specific antisera for Ia antigens have been used to determine whether specificities are of the same or different molecules.

The breeding program which was begun last year has continued successfully. Selected third and fourth generation animals have been reciprocally skin grafted, and this has led to the development of large quantities of high titered antiserum with histocompatibility specificity. Fourth generation offspring have yielded three breeding pairs of animals homozygous for serologically determined transplantation antigens, thus establishing the capacity to breed three different herds of swine, each homozygous for a different SL-A antigen. MLC assays have shown close correlation with serologically determined histocompatibility antigens. This indicates that the breeding is selective for homozygosity at a major histocompatibility complex homologous to that of other mammalian species.

Patients with various diseases are HL-A typed and associations are sought between specific antigens and disease. An interesting observation has been made in lung cancer. Patients with the antigens HL-A5 and/or W19 had a sixty percent chance of surviving disease free for one year post-therapy while patients who lacked these antigens had only a fifteen percent chance for surviving one year disease free. Attempts are being made to extend this study prospectively in order to confirm the observation.

The myeloma protein of a young patient with myeloma has been isolated and idiotype antisera prepared. These antisera have been used to monitor quantitative levels of myeloma protein in the patient as a means of monitoring his therapy. The patient continues in clinical remission, but low levels of idiotype are still detectable in his serum. This idiotype could not be detected in the serum of forty-seven other patients with IgA myeloma nor could it be detected in the patients to parents or for siblings.

A study of the immunotherapy of melanoma has been in progress for several years. A study of patients with stage III disease has been terminated after thirteen patients were placed into the chemotherapy arm of the study and sixteen into the immunotherapy arm. There were no striking significant differences between either of these therapies alone.

The research program of the Metabolism Branch is directed toward two major goals: to define host factors that result in a high incidence of neoplasia and to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. A broad range of immunological investigations are carried out in patients with malignancy, in patients with immune deficiency diseases and a high incidence of neoplasms as well as in experimental animal systems to determine the immune processes important in surveillance against neoplastic cells, to define defects in the immunological system that result in an increased incidence of neoplasia, and to develop techniques for the detection of circulating tumor associated antigens that may be of value in the diagnosis of malignancy.

Sensitive quantitative radioimmunoassays for oncofetal proteins such as alpha-fetoprotein (AFP) and the beta chain of human chorionic gonadotropin (hCG-β) that were established in previous years have been evaluated as tests to assist in the diagnosis of certain forms of malignancy and in the monitoring of the effectiveness of therapy of different forms of cancer. The radioimmunoassay for alpha-fetoprotein used in conjunction with the assay for hCG-β was of special value in monitoring the effectiveness of therapy of certain forms of malignancy. Studies of alpha-fetoprotein in patients with benign liver disease showed that approximately 20% of them have elevated alpha-fetoprotein levels. Concentrations were more frequently elevated in disorders characterized by a prominent parenchimal necrosis, inflammation and extensive liver regeneration. Evidently, alpha-fetoprotein synthesis measures liver regeneration, a function not assessed by other clinical tests.

A significant effort over the past years has been directed toward defining the nature of the events of cellular differentiation, cellular interactions and cellular biosynthesis involved in the specific circulatory and cellular immune responses to antigens. These studies are directed at defining the factors controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo a sequential maturational process from stem cells originating in the marrow. The first step that does not require antigen but requires a central lymphoid organ involves the maturation of marrow stem cells into B lymphocytes, whereas the second phase which is either antigen or mitogen driven involves terminal differentiation of B lymphocytes into cells that synthesize immunoglobulins and antibodies. The majority of patients with immunodeficiency diseases appear to have a defect in the terminal differentiation of B lymphocytes into immunoglobulin synthesizing and secreting cells. Therefore, a technique for the study of the terminal differentiation of such B lymphocytes to define the nature of this critical process and define defects in this process in patients with immunodeficiency disorders has been developed to study the role of regulatory T cells in the control of this maturational event.

In normal individuals, the terminal B cell maturational process can be triggered by the pokeweed mitogen, the only chemical agent to cause a marked and consistent stimulation of maturation and synthesis by B cells. A number of physiological factors were shown to inhibit this maturational process: Dibutyryl cyclic AMP or factors which increase intracellular cyclic AMP such as isoproterenol and prostaglandin E_1 prevented B cell maturation; HCG-β and alpha-fetoprotein also inhibited this process. Results of preliminary studies suggest that two mechanisms are of significance in this inhibitory process. The first mechanism is a direct inhibitory action of these soluble mitogens on the B cells. B cells and T cells have equal number of receptors for phytohemagglutinin and concanavalin A, which inhibit the immunoglobulin synthesis of purified B cells. In soluble form they may interfere with immunoglobulin production by B cells by interacting directly with the receptors for these mitogens on the B cells, leading to an inhibitory signal to these cells. Also, a subset of the population of T cells can be activated by concanavalin A to nonspecifically suppress antibody production by B cells.

The studies of cell-mediated immunity using a variety of basic model systems were continued. Several of these systems were extended to study the state of immune competence of patients with neoplastic disease and/or immune deficiency disease. Four fundamentally different cellular immune functions have been assessed in vitro by different techniques: (1) Proliferative response of lymphocytes following stimulation with mitogens, specific antigens, and allogeneic cells; (2) The production of biologically active soluble mediators (lymphokines) secreted by activated lymphocytes; (3) The cytotoxic killer cell activity of both immune and non-immune lymphoid cells and macrophages; and (4) The in vitro responsiveness of mononuclear leukocytes (monocytes and macrophages) to various lymphokines and other biological mediators such as activated serum complement components.

One leukocyte function receiving intensive investigation is the cytotoxic effector capacity or killer activity by which lymphoid cells destroy various tumor cells and other tissue targets. To investigate the basic mechanisms of cytotoxic reaction, two model systems (antibody-dependent cellular cytotoxicity or ADCC and mitogen-dependent cellular cytotoxicity or MDCC) have been employed. ADCC is a cytotoxic reaction in which target cells coated with specific antibody are killed by non-immune effector lymphoid cells. The identity of the effector cell-mediating ADCC has been the subject of considerable controversy with little agreement between the various laboratories involved in these studies. We have found that many cell types possess the capacity to kill various antibody coated target cells. This activity depended upon the nature of the target cell under attack and the type of antibody utilized in the system.

Mitogen activated cellular cytotoxicity is another model system for cell-mediated target cell killing which utilizes a mixture of effector cells, target cells and various plant lectins to develop cytotoxicity. Lectins such as phtohemagglutinin, concanavalin A and pokeweed mitogen are effective in inducing the killer cell reaction to a variety of target cells. These assays are very dependent upon the type of target cell studied and specific lectin employed. It was found that the lectin provides a specific link between the effector cell and target cell so that the cytotoxic reaction can proceed. The lectin provides a certain degree of specificity in these reactions for both the effector and target cell must have a receptor for the lectin in order for the linking to proceed normally.

Another major area of investigation involved mechanisms of immunoregulation. One of the most striking examples of physiologic immunoregulation occurs during pregnancy as demonstrated by the absence of immunologic attack by maternal cells on the histoincompatible placenta and fetus. It was found that human chorionic gonadotropin (HCG) is a potent immunosuppressive compound. HCG in concentrations found at the junction of the placental and uterine vessels inhibited proliferative and cytotoxic effector cell activity of human peripheral blood lymphocytes. The most profound inhibitory activity on proliferative responses was noted in specific antigen induced lymphocyte responses and in mixed lymphocyte reactivity, while nonspecific mitogen induced reactivity was inhibited by higher HCG concentrations. In addition, the cytotoxicity effector function of lymphoid and nonlymphoid cells was also blocked by HCG in ADCC and MDCC assays. The immunosuppressive effect of HCG was completely reversible so that cells washed free of the hormone regained normal functional capacity. Thus, HCG possesses many of the properties of an ideal immunosuppressive agent for pregnancy. It works most efficiently in inhibiting specific antigen induced immune responses; it is an efficient immunosuppressive only in concentrations found at the site of maximum desired effect (the placenta-uterine interface); its immunosuppressive effect is completely reversible so that generalized maternal immunocompetence is not impaired; and it suppresses both the recognitive-proliferative phase and also the cytotoxic effector phase of cellular immunity.

Studies of patients with immunodeficiency disease and a high incidence of malignancy, including patients with the Wiskott-Aldrich syndrome, hypogammaglobulinemia, ataxia telangiectasia and intestinal lymphangiectasia are continuing with the ultimate goal of correction of the immunologic defect in these patients and gaining insights into the role of immune processes in prevention and control of malignant disease. The Wiskott Aldrich syndrome is an X-linked immunodeficiency disorder with a very high incidence of infections with all classes of microorganisms and in addition the highest rate of malignant disease of any of the immunodeficiency diseases. Intensive study of these patients has demonstrated pronounced defects in both humoral and cellular immunity characterized by clinical anergy and defective antibody production. In spite of these pronounced clinical immune defects, our studies have demonstrated that the effector arms of their immune system as assayed in vitro are substantially normal. One of the most striking features of this syndrome is the very high incidence of lymphoreticular malignancy found in these patients.

In studies on proline transport, biosynthesis and metabolism, it was shown that there are two forms of P5C reductase (p5CR) activity in mammalian cells. Adult rat liver has predominantly $P5CR_i$ whereas fetal liver has 2-3 times the specific activity of P5CR and the enzyme is predominantly $P5CR_s$, the conversion from $P5CR_s$ to $P5CR_i$ with development so that the ratio is about 1:1 at 3-4 weeks of age. Rapidly proliferating cells in tissue culture have only $P5CR_s$. This applies to all epitheloid and fibroblast cells tested. In lymphocytes undergoing phytohemagglutinin-induced blastogenesis, total P5CR activity increased 15-fold and the activity converts from $P5CR_i$ to $P5CR_s$. These observations suggest that accompanying cell proliferation, the activity of P5CR increases but the increased activity is sensitive to feedback inhibition by proline.

The genetic defect in type II hyperprolinemia has been characterized as an absence of P-5-C dehydrogenase (P5CD). Carriers for the defect may be identified by studying P5CD activity in peripheral leukocytes. The transmission of the defect is autosomal recessive. Since patients with the disorder excrete 3-OH-P-5-C as well as P-5-C, the possibility exists that these respective intermediates in the degradation of hydroxyproline and proline may be dehydrogenated by a single enzyme. Radioactively labeled 3-OH-P5C was enzymatically synthesized, purified and assayed for conversion of 3-OH-P5C to gamma-OH-glutamate in normal and type II hyperprolinemia fibroblasts. The activity in extracts from normal fibroblasts was linear with increasing time and extract protein and was inhibited by P-5-C. In contrast, 3-OH-P5C dehdrogenase activity was undetectable in type II hyperprolinemic fibroblasts. These studies suggest that 3-OH-P5C and P5C are oxidized by a single enzyme which is absent in type II hyperprolinemia.

The Laboratory of Immunodiagnosis was initiated in December, 1974. During this past year, an increasing emphasis is being placed on the immunodiagnostic implications of this work and on the development of new procedures for the immunodiagnosis of cancer and for reliable monitoring of disease in tumor-bearing individuals.

Extensive studies have been performed with C57BL/6 mice inoculated with murine sarcoma virus (MSV). Most stocks of MSV cause temporary tumor growth in adult mice and then regression. Stocks of MSV have also been found which reliably produce progressive tumor growth. It has, therefore, been possible to compare the patterns of immunity in regressors and progressors. In the regressors, the antibody level started to decline after 60 days, but in the progressors, the levels remained high. With regressor stocks of virus, high cell-mediated cytotoxicity could be detected in a four-hour ^{51}Cr release assay at about 14 days. Reactivity then rapidly declined to low levels after 20 days. In progressors, the same kinetics of response were seen but the levels of cytotoxicity were significantly lower.

A variety of assays of cell-mediated cytotoxicity is available for study. Efforts have been made to understand the interrelationships among these assays and to determine the assays most useful for monitoring of cell-mediated immunity. In the MSV tumor system, the ^{51}Cr release assay, an ^{125}IUdR release cytotoxicity assay and the visual microcytotoxicity assay were compared. Essentially the same kinetics of response were seen in all three assays. In a rat tumor model, using a chemically induced breast adenocarcinoma tumor system in Fisher rats, there does appear to be substantial differences in the cellular cytotoxicity results obtained using each of three different techniques. The ^{51}Cr assay appears to measure early membrane damage by cytotoxic lymphocytes, whereas a longer incubation time (24-48 hours) is required for release of the other two isotopes.

Extensive studies have been performed on the cryopreservation of effector cells and of target cells in these systems. This has proven extremely useful in standardizing the assay and minimizing day-to-day variations.

An apparent paradox in the MSV tumor system and other syngeneic systems has been the short-lived cytotoxic cellular immune response in contrast to the long lasting *in vivo* resistance to tumor challenge. It has been demonstrated that challenge with tumor cells carrying the appropriate antigens resulted in rapid appearance of a secondary cytotoxic response. High levels of cytotoxic reactivity could be consistently detected. Studies of the secondary response to FBL-3 have shown that if peaked earlier (days 3-5), the reactivity was higher and persisted longer than in the primary response. The secondary cytotoxic response in both the MSV and FBL-3 systems was found to be T cell dependent. Inoculation of immune lymphoid cells with tumor cells also resulted in the generation of an in vitro secondary cytotoxic response in both of these tumor systems. Strong cytotoxicity could be demonstrated even at low effector:target cell ratios.

A major concern in these studies has been the specificity of the reactions and the relationship of the antigens detected to transplantation antigens which can induce resistance to tumor growth. In vivo tumor transplantation experiments showed that only the F and FMR antigens could function as transplantation antigens. Testing by the ^{125}IUdR release assay, the specificity of the primary cytotoxic response was also mostly against F and FMR antigen, with very little reactivity against non-FMR antigens. Studies of the MSV system have indicated that the antigens detected were not directly related to MSV or FMR leukemia antigens, but rather to mouse endogenous type C viruses. Many mouse tumor cell lines in vivo and in vitro have been shown to contain this antigen and other markers of endogenous viruses. Infectious endogenous viruses could be isolated from most of these cells; they could infect mouse embryo fibroblasts or xenogeneic cultured cells. The endogenous virus associated antigens have been found to also act as transplantation antigens in the MSV system.

Cell-mediated immunity could also be detected by the lymphocyte stimulation assay. Low levels of stimulation were seen when lymphocytes from mice inoculated 14 days before with MSV were cultured with mitomycin treated Rauscher leukemia cells. Suppressor cells in the spleens of these tumor bearing mice were shown to partially inhibit this stimulation by tumor cells, inhibited the in vitro generation of cytoxic cells, and also depressed reactivity to mitogens and to allogeneic cells. Macrophages are responsible for the suppression. In addition to depressing proliferation of lymphocytes, they have also been shown to have potent inhibitory effects on the in vitro growth of tumor cells. These growth inhibitory effects were nonspecific in that a wide variety of tumor cells were equally affected. Potent suppressor cells could be induced also by growth of a variety of other mouse tumors and by inoculation of *Corynebacterium parvum*. In addition to suppressor macrophages, it was found that some tumor cells themselves could inhibit lymphocyte stimulation reactions, this being mediated by cell-free filtrates derived from the tumor cells. Current evidence suggests that it is due to an infectious agent, presumably a virus. Both the suppressor cells and the inhibitory factors within tumors may account for some of the immunodepression associated with tumors, and may also explain many of the negative cellular immunity reactions seen in tumor systems. In a Gross virus-induced tumor system in rats with progressive tumor growth, no positive lymphocyte stimulation was seen. These tumor bearing rats were also found to have depressed responses to mitogens. As with the MSV system in mice, this lack of stimulation in progressor rats was found to be due to suppressor cells. After removal of suppressor cells on adherence columns, positive stimulation by tumor cells could be detected. In addition to the suppressor cells, high concentrations of (C58NT)D tumor cells themselves were shown to inhibit lymphocyte stimulation. This effect seems to be the same as that described above for mouse tumor cells. Both types of inhibitory phenomena have also been found in the rat mammary tumor system.

Lymphoid cells from many normal rats and mice of a variety of inbred and random bred strains were found to have reactivity in the ^{51}Cr release cytotoxicity assay against several syngeneic and allogeneic tumors. In both rats and mice, peak reactivity was seen in young animals. Older animals had little or no reactivity. In the rats, this activity could be shown to be independent of T cells and macrophages. In the mouse, very high reactivity was seen with cells from athymic, nude mice, suggesting that the mouse natural reactivity was also independent of T cells. In addition, treatment of cells with anti-Θ plus complement had little effect, and removal of macrophages also did not inhibit reactivity. No cell surface markers could be found on the natural cytotoxic cells, and it has been concluded they represent a novel subpopulation of lymphoid cells, tentatively designated N cells.

Of central importance in studies of tumor immunity is the relationship of the results obtained in in vitro assays to the in vivo response to tumor growth. An adoptive transfer system has, therefore, been established to determine the effect of various cell populations at various times after immunization in resistance against tumor growth. Cells from regressor mice from 14-40 days after MSV inoculation protected recipients completely from MSV tumor induction. Cells at 14 days after MSV also protected very well against challenge with a Rauscher virus-induced tumor. Cells from progressor mice at 14 days also gave some protection against MSV and tumor cells. Adoptive transfer experiments have also been prepared in the FBL-3 tumor system. Although lymphocytes from progressor mice had some cytotoxic reactivity, it was 3-10 times lower than that of cells from regressor mice. When these lymphocytes were used in adoptive transfer experiments, the regressors' lymphocytes gave 90% protection against tumor (FBL-3) challenge, whereas the progressors' lymphocytes only gave 40% protection.

A series of assays for measuring the general cell-mediated immunocompetence of cancer patients have been established: enumeration of the percent of T cells in peripheral blood by rosette formation with sheep erythrocytes, cytotoxic reactivity against human lymphoid cell lines, and lymphocyte stimulation by mitogens, antigens and mixed lymphocyte cultures. Continued use of cryopreserved cells in these assays have standardized the day-to-day variation to a large degree. Assays for rosette formation with sheep erythrocytes performed at 29°C were particularly discriminatory between cells from cancer patients and controls. A large proportion of patients with carcinomas, sarcomas, and melanomas were found to have significantly depressed levels of rosette forming cells (RFC). In case of localized disease (77%) and in metastatic disease (100%), levels of RFC were significantly below normal controls. In contrast, only 10% of normal individuals had significantly depressed percentages of RFC.

The ability of human T cells to form rosettes with sheep erythrocytes has led to a method for isolation of T cells from peripheral blood leukocytes. Fractions with high yield and purity have been obtained. In assays of lymphocyte stimulation, reactivity to the mitogens PHA and concanavalin A, and to the antigens PPD and tetanus, and to allogeneic leukocytes has been completely restricted to the T cell fraction. Different results were seen with a staphylococcal antigen; this produced strong stimulation of non-T cells as well as T cells. It seems likely that this antigen represents one of the first strong stimulants for human B cells or other non-T cells. In assays of cytotoxicity against human lymphoid cell lines, both T and non-T cells were also found to be reactive. In addition to the rosette assay, cancer patients, especially those with larger tumor burdens, tend to show poor response to mitogens and to alloantigens in the mixed lymphocyte culture. These assays are currently being used to monitor patients on immunotherapy and to determine if they are helpful in the clinical evaluation of such patients.

In the leukocyte migration inhibition assay, the leukocytes of patients with carcinoma of the breast, malignant melanoma, carcinoma of the lung, and Ewing's sarcoma have been shown to be specifically inhibited by 3M KCl extracts of autologous and allogeneic tumors of the same histological type. Little cross-reactivity of patients with other types of cancer or of normal controls has been seen.

A major effort has been made to study stimulation of cancer patients' lymphocytes by autologous tumor cells and by membrane extracts and 3M KCl extracts of autologous tumors. In approximately 50% of the patients tested thus far, stimulation has been seen with tumor materials and not with normal control tissues. The membrane extracts appeared particularly promising in that very low concentrations gave positive results.

Patients with cancer have been shown to have delayed hypersensitivity reactions to extracts of tumor cells and of some tissue culture cell lines derived from tumors. A prospective study of reactivity of African patients with Burkitt's tumors is being performed with extracts from a cell line derived from a Burkitt tumor. Reactivity to this extract has correlated well with clinical status. Before therapy, only patients with small localized tumors have reacted. When patients have been successfully treated and then tested in remission, a high proportion have been reactive. Patients failing to go into remission or patients with early relapse have tended to be unreactive although they were not anergic. The skin reactivity has been correlated with serological studies of antibodies to EBV associated antigens, and a very significant inverse correlation between skin reactivity to the cell line extract and antibodies to EBV nuclear antigen has been found.

In the Laboratory of Cell Biology, studies continue on the usefulness of the alpha-fetoprotein (AFP) marker in human disease: AFP and serum chorionic gonadotropin (hCG) markers have been used in a prospective manner to gauge effectiveness of treatment in hepatocellular carcinoma and embryonal cell testicular carcinoma; AFP levels are high in some forms of tissue damage and appear to be related to liver degeneration.

BALB/c mice were used to determine the pathogenic potential of Dl-MuLV. When injected into BALB/c mice 1 day after neonatal thymectomy, 30% of the inoculated mice developed RCN-B in 5 months, whereas none of the uninoculated controls did. From the spleen and lymph node extracts of all RCN-B-bearing mice tested, B-tropic type C viruses were isolated in high titers. Only low titers of N- or B-tropic viruses were isolated from those thymectomized mice inoculated but nontumorous, whereas only N-tropic viruses were detected in the uninoculated thymectomized mice. No virus was isolated from the nonthymectomized untreated controls.

Using high resolution electron microscopy and a combination of preparative techniques, it has been possible to gain important new information about the structure of intracisternal A particles (IAP). Through the use of critical point drying plus platinum shadowing, it has been possible to view unsectioned IAP as "whole mount" preparations with three dimensions. Our data indicate that isolated IAP have an average diameter of 85 nanometers, they are essentially uniform spheres as indicated by the shadow length, and they consist of two concentric shells surrounding a hollow core. It can be demonstrated that numerous single IAP have an apparently intact outer shell. The fact that we can isolate IAP that do not exhibit defects in the outer shell strongly suggests that a certain proportion of these particles are released from the membrane into the cisternal spaces of the endoplasmic reticulum.

In tracing the morphologic sequence of events preceding the development of IAP, it was discovered that crystalloid aggregates contiguous with the endoplasmic reticulum appear first in the fertilized egg. These structures increase in size and number until the morula stage. During the blastocyst stage, crystalloid units often form densely staining plaques. As these crystalloids continue to develop, budding and complete IAP begin to appear. By the time the embryo reaches the outgrowth stage, these crystalloids and plaques can no longer be detected, although IAP can still be found.

It is known that human mononuclear leukocytes (HML) possess IgG surface receptors which may be involved in phagocytosis. Such receptors can be detected by the ability of HML to form rosettes with IgG-coated erythrocytes. During phagocytosis this IgG receptor activity disappears. When HML are exposed to a chemotactic gradient across a millipore filter, these cells line up at the filter surface and long pseudopods are thrust into the filter pores. In this condition the tail of the HML cell farthest from the chemotactic stimulus does not form rosettes with IgG coated erythrocytes. This loss of receptor activity is not due to inhibition by chemotactic factor since exposure of cells to this factor on both sides of the filter chamber does not cause similar impairment of rosette formation. These results indicate that HML lose their IgG binding receptors at the trailing end of the cell in response to a chemotactic stimulus. The mechanism by which these changes occur could be based on internalization, rearrangement of migration of receptors or a combination of such events.

In the last year the levan binding myeloma protein system has been intensively investigated. Fourteen proteins have been collected and characterized. These have been divided into two groups: a large group of proteins that bind β2,1 linked fructosans and a smaller group with specificity for β2,6 linked fructosans. The individual and cross-specific idiotypes of these proteins were studied. Each protein has an individual idiotype and one or more cross-specific determinants. The large number of these determinants suggests a degree of variability of closely related proteins that may be the result of a form of somatic variation.

The crystallization of the Fab fragment from the phosphorylcholine binding myeloma protein McPC603 and its general structure at 4.5 Å resolution have been previously described. As a result of the determination of the variable region amino acid sequence and the further refinement of X-ray data, a high resolution model of the antibody combining site has been constructed. A cleft has been found at the tip of the molecule which is formed by the three hypervariable regions of the heavy chain and two of the three from the light chain. This surface seems to be universal among antibodies and thus provides the structural basis for antibody function and homology. The hapten, phosphorylcholine, has been found to form hydrogen bonds with a tyrosine residue in the first heavy chain hypervariable region and an arginine in the second heavy chain hypervariable region.

Preliminary comparative sequencing studies of five phosphorylcholine binding myeloma proteins have previously been reported. These studies have been extended, and the heavy chain variable region determinations on all of the proteins have been completed. This comparison has permitted an assessment of the degree of variability among proteins demonstrating the same hapten binding specificity. The differences observed among these proteins have also provided a unique opportunity to assess the possible mechanism involved in the generation of antibody diversity.

Direct modification of cell surface carbohydrates was achieved by certain metabolic modifiers of the biosynthetic pathways of glycoproteins and glycolipids. Such modifiers as the fluorinated N-acetyl-glucosamine, closely resembling N-acetyl-glucosamine, a common metabolic intermediate, were studied. Replacement of hydrogen by fluorine results in little steric change, but the vastly different electronegativity has a definite effect on the interaction of a fluorine containing molecule with an enzyme. It was found that in extracts of mouse cells, addition of nontoxic amounts of the fluorinated N-acetyl-glucosamine, the enzyme dependent synthesis of the nucleotide sugars uridine N-acetyl-glucosamine pyrophosphate and cytidine-acetylneuramininyl monophosphate was greatly inhibited. These neucleotide sugars are key intermediates in sialic acid biosynthesis. The metabolic method and the clarification of the mechanism opens important new ways to study the biologic consequences of alteration of sialic acid residues in the highly hydrophilic glycoproteins on the outermost cell surface.

CLINICAL RESEARCH

The area of clinical research includes: cancer surgery, radiotherapy, work on skin cancer, and general cancer diagnosis. The branches covered here are: Surgery, Radiation, Dermatology, and the Laboratory of Pathology. The program of the Metabolism Branch was reported under Immunology, and the Diagnosis contract program is included with Contract programs. The Image Processing Unit is organizationally part of Diagnosis Branch, but its work is intramural; therefore, a short report is included in this group of summaries.

On July 1, 1974, the Image Processing Unit was transferred from the Division of Cancer Treatment to the Division of Cancer Biology and Diagnosis. The work was completed on the redesign, debugging and reconstruction of the first model Grain Counter. Construction of the four field models is about to be let on a contractual basis. The Grain Counter/Densitometer was originally intended to go beyond simple counting of autoradiographic grains in microscopic images. Its present functioning capabilities include the ability to automatically separate grains for each cell into nuclear and non-nuclear categories, to measure the area of the nucleus of each cell and its optical density. Individual results on running totals for each of the 16 user assignable classes of cells are kept automatically. A variety of shape related measures are also obtainable. By changing one or more filters in the array of six computer controlled filter changers, it is possible to quantitate cytoplasm, various inclusions and/or extracellular components. Additionally, the Grain Counter may function as an "electronic micrometer," a thickness measuring device, etc. Without any additional hardware, it is possible to convert the instrument into a simple "shape"/texture analyzer, an analyzer of double labeled material, or a cell locator. Provision for additional expansion of capabilities along lines required by individual users exists. Additional functions may be embodied on a circuit card for direct plug-in to the existing electronics without redesign or rewiring.

The Automatic Cell Analysis project involves the construction of a fully computer controlled microscope, a special purpose processor and a controlling mini-computer along with necessary monitor and debugging software, Real Time Picture Processor (RTPP), and of an artificial intelligence-like system resident in the DCRT PDP-10, embodying a procedural description language and facilities for on-line modeling of cell descriptions and control facilities for RTPP. The system is intended to perform real time measurements and characterization of living cells as well as fixed and stained material. In fact, the ability to track a cell and its direct descendants through several successive cell cycles is a major reason for constructing a system of such complexity.

Over the past decade the Surgery Branch at the National Cancer Institute developed an international reputation for excellence in the radical surgical approach to cancer treatment. The Surgery Branch has been a major center for the development and application of new radical surgical operations. The development and application of the craniofacial exenteration, major pelvic exenterative surgery, and major head and neck resective and reconstructive surgery has been a hallmark of the Surgery Branch's activities in the past.

Last year was a year of transition for the Surgery Branch. On July 1, 1974, Dr. Steven A. Rosenberg became Chief of the Surgery Branch, replacing Dr. Alfred Ketcham, who had held that position for the past 17 years. With Dr. Rosenberg came a change in philosophy and emphasis within the Surgery Branch.

The realization that two-thirds of patients already have disseminated disease at the time they first consult a surgeon requires that the surgeon gain expertise in the control of disseminated disease as well as the local control of cancer. Towards this end, the clinical and laboratory efforts of the Surgery Branch will be devoted to the development of innovative immunologic and other approaches to the treatment of malignant disease to be used as surgical adjuvants. The Surgery Branch will also place emphasis on its involvement in the administration of these adjuncts in many of its protocols.

In keeping with this philosophy the Surgery Branch this year submitted several protocols involving the combined modality treatment of cancer, joint participation of the Surgery, Immunology, Pathology, and Biology Branches of the NCI, as well as Radiation Oncology and the Chemotherapy Branch. The Surgery Branch is involved in an extensive review of its clinical activities in an attempt to develop innovative combined modality treatment protocols for a variety of diseases.

Shortly after the summer a major reorganization of the Surgery Branch clinics was undertaken. All patients were assigned to individual clinical associates or senior staff and continue to see that same physician during his entire tenure at the NIH. The surgical training program was also modified. Surgical Grand Rounds were instituted to enable invited speakers, both from within and outside of the NIH, to give formal presentations of their work. This has provided a forum to keep the Surgery Branch abreast of recent developments in surgery and related fields. A conference is also held weekly immediately preceding the Surgical Grand Rounds to discuss the preoperative cases for the following week. Chief's Rounds was organized on Tuesday afternoons. During these rounds, Dr. Rosenberg meets with the senior staff and residents on the ward service to discuss individual patient management problems in more detail than is possible at other sessions. A daily meeting between the Surgery Branch and the Radiology Department to review patient x-rays has also been instituted.

The clinical research program has been strengthened by the creation of a medical service in the Radiation Oncology Branch, institution of a medical oncology fellowship program, and an increased emphasis on laboratory investigation. The Ewing's sarcoma program has become a model example of the combined modality treatment concept. Systematic evolution in the manner of integrating radiation therapy and chemotherapy has produced a five-fold increase in the number of long-term, disease-free survivors.

A comprehensive review of ten years experience with chronic lymphocytic leukemia was completed during the year. Carefully documented information on patients with active, symptomatic chronic lymphocytic leukemia using total body irradiation as the principle method of treatment has demonstrated that the natural history of disease can be modified by therapy. One-third of patients experienced essentially complete clinical and hematologic remissions, return to a normal performance status, recovery from hypoimmunoglobulinemia and absence of infectious complications, and significant prolongation of survival.

The long-term data collection on patients treated for Hodgkin's disease between 1965 and 1969 has continued to yield insight into both the disease itself and the delayed consequences of treatment. The collaborative study of the non-Hodgkin's lymphomas has contributed in a major fashion to understanding of these protein diseases.

The staff of the Radiation Oncology Branch has continued to provide extensive advisory and collaborative support to other intramural program areas. As usual, thousands of non-clinical radiation exposures of biological materials and animals were performed, often requiring extensive planning in order to provide high quality support to the respective research efforts. Two hundred and seventy patients were seen formally in consultation at NIH and approximately 150 of these were treated with radiation therapy. Extensive telephone consultations were provided to over 100 physicians in community practice during the past year. These efforts, while not reflected in the Branch bibliography, are nonetheless viewed as a meritorious contribution.

The Dermatology Branch conducts both clinical and basic research studying the etiology, diagnosis and treatment of malignant diseases involving the skin and the host's response to these diseases.

Interesting results were obtained from the study of Sezary Syndrome, a lymphocytic leukemia of the T-cell type, characterized by intense generalized reddening and exfoliation of the skin, along with leukemic cells circulating in the blood. Three patients with cutaneous T-cell leukemia have now been treated by leukapheresis. One, who presented at the time of initial therapy, has been adequately managed for the last 18 months by leukapheresis alone, two other patients have also significantly benefited.

Lymphomatoid papulosis is an unusual disease in which, although malignant looking cells are found in skin tumors, they ordinarily disappear spontaneously. Patients with this disease were studied clinically, microscopically and immunologically. Findings have shown that the infiltrating cells in this disease are T-lymphocytes and that nuclear irregularity of the cells of the dermal infiltrates is significantly greater than infiltrating lymphocytes in delayed hypersensitivity reactions. Patients with the syndrome demonstrate normal reactivity to intradermally applied antigens and normal *in vitro* stimulation of their lymphocytes by mitogens.

Xeroderma Pigmentosum is a rare autosomal recessive dermatologic disease, characterized by pigmentary changes, telangiectasia, atrophy and tumor formation in response to even minimal sun exposure. Tumors which commonly occur in XP patients are histologically identical to those found in otherwise normal persons. A defect in repair of UV damage to DNA has been found in XP cells.

By complementation analysis of various cell lines in XP, it has been found there are at least 5 XP complementation groups suggesting that defects arising in any one can give rise to XP. The neurological abnormalities attributable to XP occur only in patients in complementation groups A and D: the neurological abnormalities may therefore be caused only by the specific mutations that are associated with these complementation groups. The complementation groups correlate with the magnitude of the repair defect as reflected in the rate of UV-induced thymidine incorporation, and complementating fibroblasts can restore biologic function to UV-damaged DNA. Long-term lymphoblastoid lines from XP patients peripheral blood have been established, making it possible to obtain large quantities of XP cells for biochemical analysis and, hopefully, enabling the characterization and purification of defective DNA repair enzyme or enzymes in XP cells. Understanding the relationship between DNA repair deficiency and tumor development in XP patients should elucidate the role of DNA repair, hopefully leading to prevention in other humans those cancers which may be due to certain DNA-repair related chemical and physical carcinogens as well as ultraviolet radiation.

Malignant melanoma is a highly malignant tumor in which production of melanin is often increased. The melanin granules in tumors are morphologically distinguishable from those found in normal cells. Melanogenesis and its regulation in normal and malignant cells is being investigated to determine the relationship of this process to melanoma formation; cells from various pigmentary mutants of the mouse have been compared to elucidate the genetic basis for control of melanin production. Melanogenesis followed a common pathway of melanosome formation; the major difference noted among the mutants was the premature termination of melanization of the granules at different stages of melanosome formation. Melanosomes from black mice contain seven proteins, six of which are of extremely high molecular weight. Melanogenesis in the mouse is dependent upon tyrosinase activity rather than peroxidase activity. Tyrosinase activity is under allosteric control and phospholipids play an important role in the expression of the enzyme's activity. Tyrosinase activity in black mice seems to be under the control of a heat-labile, low molecular weight inhibitor. Tyrosinase activity in albino mice can be demonstrated when tissue homogenates are pretreated with detergents. Finally, it has been found that some proteins of malignant melanoma melanosomes are unique and not found in normal melanin granules. These aberrant proteins found in malignant tissue could be used in developing an immunotherapeutic approach against melanomas.

In order to understand normal epidermal cells as a baseline for studying malignant epidermal diseases, such as basal cell and squamous cell carcinoma, a study was carried out on tonofilaments major components of the epidermal cell. It has been found that intact tonofilaments of bovine epidermis are extractable by a simple buffer system and can be purified by differential centrifugation and gel electrophoresis. The tonofilaments appear to be composed of seven different peptide chains. These isolated peptides have a propensity for aggregating into filamentous structures which make them accessible to study by electron microscopy and x-ray diffraction. Skin from patients with benign and malignant lesions have been studied using the same system in an attempt to detect whether there are any differences in these tonofilament polypeptide chains. Elucidation of their chemistry, structure, and synthesis might aid in determining the nature of human epidermal cancer.

Structural parameters of diseases are being studied with a wide range of optical methods comprising light microscopy, transmission electron microscopy, and scanning electron microscopy. These observations provide the most detailed descriptive morphologic data available with the current technology, give direct experimental evidence for the physiologic roles of certain cells and organelles, and help in evaluating the purity and sampling of cells or cell components being studied by other methods. The development, behavior, fate and pathology of leukocytes and epithelial cells are a primary concern, since they play an essential role in normal and diseased skin and are uniquely suited for experimental studies. It has been found that normal human peripheral lymphocytes almost invariably appear villus when fixed in suspension by standard procedures. There is little difference between T-lymphocytes and B-lymphocytes according to their surface structure, in opposition to what has been reported by others. A human tumor cell line (HBT-3) has been studied, and it has been found that during the first 24 hours after seeding cell structure is profoundly influenced in distinctive ways by kind of culture medium, the underlying substrate, and the concentration of supplemental serum in the medium. Further, Cinephotomicrographic studies in conjunction with scanning electron microscopy of the same cultured mouse cells provide the best evidence to date that surface morphology is independent of the stages of the cell cycle, that one cannot predict the mitotic behavior of cells, nor whether cells are neoplastic or benign by their cell topography.

The sensitization of human skin with dinitrochlorobenzene (DNCB) has become a means of testing the ability of humans to generate a cell-mediated immunity response. A quantitative correlation was found between in vivo skin test reactivity to DNCB and in vitro specific blast transformation. A factor is released by sensitized lymphocytes in culture which is capable of recruiting and inducing blastogenesis in lymphocytic cells from non-sensitized subjects. This particulate lymphokine is completely removed by filtration through .45 micromillipore filters.

Although the Laboratory of Pathology is heavily involved in the training of residents in anatomic pathology, and in histopathology and cytopathology services for patients throughout the Clinical Center, it also carries out a broad research program with major emphasis in viral oncology and other aspects of carcinogenesis. Noteworthy research results include:

Isolation and successful culture of a human papovavirus from a human brain tumor.

Characterization of surface receptor sites and immunoglobulin production by normal and neoplastic cells from patients with a wide range of malignant lymphomas and leukemias.

Demonstration that fiber size is critical not only for asbestos carcinogenesis but also for other particulate material including silicon carbide and certain fibrous talcs.

Demonstration of an association between H. varicellae in dorsal root ganglia and in herpetic skin lesions (shingles) in a patient with malignant lymphoma.

Determination of the Coulter volume distribution of cells from normal and abnormal cervico-vaginal specimens.

Use of flow analytic techniques to measure efficiently and accurately the blastogenic response of lymphocytes to various antigens.

Evaluation by EM of hepatic fibrosis in workers exposed to vinyl chloride.

Characterization of cell-mediated immunity in patients with retinal pigment degenerations and in patients with retinoblastomas.

Considerable progress has been made in the area of the automation of cytology. Since most of the work has been done under a contract supported program, it is reported in the contract section.

CONTRACTS PROGRAM

The Division of Cancer Biology and Diagnosis supports three separate program thrusts through contract mechanism. This includes the Immunology Program organized into Immunobiology, Immunodiagnosis and Immunotherapy; Breast Cancer Task Force with four separate areas of interest: Diagnosis, Epidemiology, Experimental Biology and Treatment; and Cancer Diagnosis Program, which consists of general Diagnosis research, Diagnostic Radiology and Cytology Automation. Cancer Diagnosis Program also includes two screening projects: Breast Cancer Detection Demonstration and Lung Cancer Screening.

The greatest effort is in the area of Tumor Immunology, managed by the office of the Associate Director for Immunology. The program consists of three subunits: immunobiology, immunodiagnosis, and immunotherapy. Although classified this way, some of the projects naturally cross these delineation boundaries. Basically, the goals are (1) using immunologic techniques, to detect agents etiologic to cancer; (2) to prevent cancer spread by immunologic manipulation; (3) improve diagnosis of cancer by developing immunologic methodology; and (4) manipulate the immune system of patients with established cancer to achieve increased survival. Specifically, research in immunobiology concentrates on identification and characterization of surface membrane components, investigation of host reactivity and development of special cell lines. Immunodiagnosis effort leans towards the search for new tumor associated antigens in human cancer, evaluation of carcinoembryonic and other fetal antigens as diagnostic tools and evaluation of serologic assays, humoral and cell-mediated immune response to tumor associated antigens. In the therapy area, the work is mostly preclinical, but limited human studies are being initiated with such agents as BCG and MER.

The Breast Cancer Task Force lost its Chairman in the departure of Dr. Berlin; Dr. Gullino was appointed his successor. This is the oldest contract program in this Division, directed toward multidiscipline approach to this very common cancer. It is comprised of four subdivisions: diagnosis, epidemiology, experimental biology and treatment, and its management is the responsibility of the Breast Cancer Program Coordinating Branch. The diagnosis program is further subdivided into detection (development, refinement and evaluation of instrumentation); immunology (development of long-term tissue cultures of mammary epithelial cells, and development of immunologic assays for breast cancer associated antigens); and diagnosis-prognosis segment investigating the hormonal markers. Epidemiology supports some programs of viral etiology and hormonal mechanisms, but its principle stress is on studies in the genetics of breast cancer. The biggest program is that of experimental biology, directing its efforts to in vitro cultivation of mammary cells, attempting to assess the biological characteristics of mammary epithelial cells, studying the pathophysiology of mammary tumors and their hormonal responsiveness. Finally, the growth of a mammary tumor, its metastasizing capacity and regression are being investigated. The treatment program emphasizes three main areas: evaluation of the relative effectiveness of current surgical methods, search for new methods of measuring cancer cell growth potential, and evaluation of various markers for monitoring therapy.

The Cancer Diagnosis Contract Program covers six broad areas: General Cancer Diagnosis, Diagnostic Radiology, Cytology Automation, Breast Cancer Detection Demonstration Projects, Breast Cancer Diagnosis and Immunodiagnosis. The last two areas were reported above, as they are not under direct administrative control of the Diagnosis Branch. The General Diagnosis area expands from lung cancer screening program to a cooperative study of the cancer of pancreas, several projects in gastrointestinal cancer, search for biochemical markers and evaluation of NMR technique for diagnostic purposes. In the field of Diagnostic Radiology, the attention was paid to improvements in X-ray imaging; specifically, the computer assisted tomography of the brain was evaluated, and the feasibility of using proton beams in tissue density measurement has been investigated. Cytology Automation Program concentrated on evaluation of sensing-screening and decision-diagnostic systems, as well as on development of new techniques for specimen collection and preparation. The Breast Cancer Detection Demonstration Projects evolved into a popular and successful program. There are 27 centers operating at capacity levels; about 150,000 women have been screened to date, the annual recall rate being about 90%. All the data are collected centrally. This project manifests the feasibility of collaborative effort between the government and private sectors.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE
SUMMARY STATEMENT
July 1, 1974 through June 30, 1975

This has been the first year that the Laboratory of Biochemistry has had its new organization in stable form. One of the exciting developments has been the growing interactions among the various sections, as well as interactions with the Laboratory of Molecular Biology, with whom we share the 4th floor of Building. 37. Journal clubs, laboratory seminars, and an increasing frequency of talks by non-NIHers have kept us in touch with each other's work, up-to-date on the outside world, and too busy to complain.

The work of the BIOSYNTHESIS SECTION (Dr. E. Shelton, Acting Head this year, while Dr. E. Kuff has been on an assignment at the Weizmann Institute of Science in Israel) has been carried out by four independent groups:

1. Dr. S. Wilson and his colleagues have continued their study of mouse myeloma DNA polymerizing enzymes. They have resolved DNA polymerase I into two forms, designated IA and IB, and have characterized these enzymes with respect to physical and enzymatic properties. In collaboration with Dr. J. D. Minna and co-workers (NHLI), it was found that when freshly isolated human cells were fused with mouse cells and grown in culture, the human genes could alter the amounts of murine type-C particle components present in the cell or released into the medium. Regulation of packaging and release of C-particle DNA polymerase may be attributed to genes on four human chromosome.

2. Dr. B. Peterkofsky and her colleagues have continued to study the basic mechanisms of collagen biosynthesis and secretion and the factors that regulate these processes in cultures of normal and transformed fibroblasts and synthesis of the collagen polypeptide took place within the cysternae of the rough endoplasmic reticulum (ER). They have now shown that 80-95% of the collagen-processing enzyme, prolyl hydroxylase, is also located, free, within the rough ER. Underhydroxylated collagen protein secreted into tissue culture medium is much smaller than procollagen, a result that suggests that underhydroxylated collagen is susceptible to enzymatic degradation in cell cultures. Further support has been gained for the conclusion that transformed 3T3 cells synthesize a reducing agent that functions in the prolyl hydroxylase reaction. Finally, it was found that di-butyryl cyclic AMP (dbc AMP) did not affect the levels of pryolyl hydroxylase activity under conditions in which the relative rate of collagen synthesis was increased and that an observed increase in proline transport caused by dbc AMP was probably not related to cyclic AMP metabolism but to butyric acid.

3. Dr. E. L. Kuff and his collaborators continued their study of mouse tumor-associated type-A intracysternal particles. Three main structural proteins of A-particles contain repetitive sequences and the amino terminal residue of all three proteins is argenine. The repetitive sequences appear to be connected linearly through peptide bonds. In an attempt to examine the A-particle genome, a poly(rA)-containing RNA was isolated from purified A-particles and it was found to direct the in vitro synthesis of a 30,000 MW protein. A study of the expression of A-particles and C-particles (in collaboration with Drs. Wilson and Orenstein of this Section) induced by treatment of N-4 cells with IUdR and dexamethasone showed elevation of C-particle markers but no increase in A-particle markers. This result provides

evidence of independent control of the expression of C- and A-particle genomes.

4. Dr. E. Shelton and her collaborators have continued to study the structure of macromolecules and in collaboration with Dr. R. A. Lazzerini (NINDS) have shown that the shape of vesicular stomatitis virus is similar to that of plant rhabdoviruses. Previously observed differences in shape are probably due to differences in viral membrane constituents derived from host cells. In collaboration with Dr. R. Switzer, (Dept. of Chemistry, U. of Illinois) it has been proposed that the enzyme, phosphoribosylpyrophosphate synthetase is made up of five subunits in asymmetrical quarternary arrangement. In a second project aimed at detection and characterization of cell interactions in the immune response, it was found that, in culture, plasmablasts and mature plasma cells formed very prominent, long-term associations with macrophages; this might explain the observed absence of plasma cells in blood and lymph _in vivo_. In many cultured plasma cells, no specific antibody could be detected, a phenomenon that may have a bearing on the synthesis and secretion of specific antibody by individual cells. Comparison by scanning electron microscopy of the surface morphology and cell interactions between lymphocytes and macrophages _in vivo_ and _in vitro_ showed interactions to be present in both environments, but the surface of the macrophage was profoundly altered by contact of the cell with the foreign substrates, Milliore filter and glass.

In the BIOCHEMISTRY OF GENE EXPRESSION SECTION (Dr. E. Thompson, Head), studies on rat hepatoma x mouse fibroblast hybrids indicated that glucocorticoid receptors are functionally different in the two cell types. This suggests an active role for physically similar but subtly different receptors for steroids that induce cell-specific responses. In somatic cell hybrids of the same type, the two non-contact inhibited parents combined to give contact inhibited hybrid progeny. Contact inhibited behavior correlated with a full complement of chromosomes from both parents. When injected into "nude" mice, the contact-inhibited cells showed a marked delay in tumor production, and the recovered tumor cells had a reduced chromosome number.

It was found that L cells may have a receptor for 25-hydroxy cholesterol. Specificity and characterization studies are underway.

Biopsies from over 100 patients with breast cancer have been examined for receptors for estrodiol, dihydrotestosterone (DHT), progesterone and dexamethasone. A new assay method has been developed, permitting the clear delineation of DHT receptor from the serum-carrier protein, sex steroid-binding globulin. Finding androgen and progestin receptors distinct from estrogen receptors in these malignant cells suggests a biological role for the hormones in the disease. A protocol for a prospective study to test therapy based on these findings has been prepared.

The major findings and accomplishments of the CELL PHYSIOLOGY AND ONCOGENESIS SECTION (Dr. K. Mifflin, Head) are as follows: Dr. Fox and Mr. Price have succeeded in cultivating sheets of human epidermal cells derived from skin and gingiva. In addition, Dr. Parshad has collaborated with this effort in producing pure epithelial cultures of human prostate cells that are devoid of fibroblasts. These cultures, which can be obtained regularly and consistently are fundamental to the study of the formation of carcinomatous tumors in humans. With these cultures (in collaboration with Dr. J. K. Selkirk) the metabolism of benzo(a)pyrene has been demonstrated and an unusual spectrum of quinones, epoxides, and arylhydroxides has been isolated, identified, and quantitated. Metabolism of this compound by cultured human cells has not been previously reported and represents an advance toward the goal of developing an *in vitro* system for studying chemical carcinogenesis in humans. It is believed that in order for carcinogenesis to occur, the procarcinogen must be metabolized to an active intermediate that results in effecting the neoplastic transformation. It is interesting that human cells *in situ* metabolize this compound but that cultured human cells do not.

Dr. Fox is conducting a study to identify and quantitate subjective estimations of neoplastic properties, using cultured human and rodent cells. At present, measurements are being made of changes in cell geometry, in order to develop criteria by which the morphology of transformation can be used to improve cytodiagnostic methods for human cancer.

Dr. Fox is studying regulation of glycolysis in cultivated cells as a model system for studying human tumor metabolism. Fetal tissues, cancer tissue, and tissue cultures have the common characteristic of converting glucose to lactic acid. In tissue cultures the lactate so produced is not further metabolized. He has developed a system for analysis of metabolites from cells that will quantitatively measure picamole amounts of metabolites from whole cells and culture media.

Dr. Tucker has continued studies on the susceptibility of neoplastic cells to BCG-activated macrophages and has found that the degree of susceptibility of established cell lines correlates with their tumorigenicity. Additional studies have determined that BCG-activated macrophages can be used as an *in vitro* assay. Other *in vitro* assays of neoplasia, such as selective growth in agarose, (with Dr. Sanford and Mr. Jones) and the 24-hr-colony morphology (with Ms. S. Handelman and Dr. Sanford) also correlate with tumor development *in vivo*.

In attempts to control the karyotypic instability of mouse cells in culture that may be a causative factor in "spontaneous" neoplastic transformation, Dr. Sanford and co-workers (Ms. Handleman, Mr. Price, in collaboration with Dr. Parshad) have confirmed and extended previous observations that fetal bovine serum (FBS), as compared with horse serum (HS), promotes karyotypic stability in freshly isolated mouse cells. The higher zinc level in FBS is not the cause. The large molecule fractions of HS is associated with an increased shift from diploidy and appearance of abnormal chromosomes. Decreased O_2 concentration (1% O_2, as compared with atmospheric 18%) promotes karyotypic stability.

In studies by Dr. Sanford and Ms. Handleman, six morphologic features of cultured cells have been found to show high coefficients of correlation with the neoplastic state, suggesting a relationship. A method for measuring one of these, parallel orientation, has been developed by Ms. Handleman and is being evaluated for computer programming. Three of the other morphologic features are being quantitated by Dr. Fox. The objective of this study is to provide quantitative methods for cytodiagnosis of the neoplastic state in cultured cells.

Cell surface features have been considered indices of neoplastic transformation. Drastic changes in surface morphology reportedly occur as cells progress through the mitotic cycle. Dr. Sanford and Mr. Jones (in collaboration with Dr. B. Wetzel) have examined cell surfaces during the mitotic cycle by scanning electron microscopy and cinephotomicrography in a clone of normal and derivative neoplastic cells. The cinematographic instrumentation for this project was developed by Dr. Fox. The conclusion drawn from this study is that cell surface morphology does not change predictably as these cells progress through the mitotic cycle and is not significantly altered in neoplastic transformation.

Dr. Taylor made significant advances in his studies on cell growth and neoplastic transformation *in vitro*. By rapid, continuous subculture of cloned cell populations, the relationship of cell-cell contact and neoplastic transformation was assessed under conditions which minimized cell-cell contact and prevented cultures from reaching saturation density. Nevertheless, both cloned populations underwent neoplastic transformation, although one cloned line exhibited a significant delay in onset of tumorigenic capacity. In related studies, susceptibility of these cell populations to activated macrophages (in collaboration with Dr. Tucker) and cytologic alterations observed during the course of the study (in collaboration with Dr. Sanford) correlated well with *in vivo* tumorigenesis assays. In studies on the influence of low (1-2% v/v) oxygen on colony development (growth), several cell inoculum sizes and graded oxygen concentrations were used to establish an inverse relationship between oxygen dissolved in the medium (pO_2) and colony development. Enhanced colony development of adult human cells showed that improved plating efficiency with "low oxygen" is not restricted to cells of fetal or embryonic origin. Studies with peptone dialysate (PD) and autoclavalbe serum-substitute, suggest that PD is not a source of nutrients, does not function as a protective agent, and does not effect attachment to the growth surface. The active component(s) probably is a low molecular weight peptide, and purification is in progress.

Dr. Gantt and co-workers are investigating the biological function of cellular nucleic acid methylation and its relationship to malignant transformation and RNA tumor virus life cycle. They have partially purified the N^2-guanine RNA methyltransferase associated with avian myeloblastosis virus and determined the specific site of phenylalanine transfer-RNA which is methylated. They are purifying selected RNA methylases from chick embryo cells to acquire purified antibodies for monitoring specific methylases implicated in differentiation and malignant conversion. With regard to DNA methylation he has found that the distribution of 5-methylcytosine is considerably higher in the repetitive sequences of chick DNA. This information may be useful in understanding the biological role of eucaryotic DNA methylation.

This year the CELLULAR REGULATION SECTION (Dr. Goldberger, Head) has cleared the decks of old work and made real headway in a previously uncharted sea of adventure. We have been writing up our final studies on regulation of histidine biosynthesis and initiating studies on regulation of gene expression by hormones in higher organisms.

Our work on histidine biosynthesis includes isolation of mutants of a new class, in which mutations in the gene that specifies the first enzyme for histidine biosynthesis cause constitutive expression of the histidine operon; and studies that show that the first enzyme for histidine biosynthesis binds specifically to the DNA of the regulatory region of the histidine operon *in vitro*. These studies have provided strong support for our previous conclusion that the first enzyme for histidine biosynthesis plays a direct role in autogenously regulating expression of the histidine operon.

The new area we have been studying is the estrogen-mediated regulation of egg yolk protein synthesis in the rooster. We found that egg yolk proteins are normally synthesized as a large precursor, vitellogenin, in the liver of the laying hen, but roosters synthesize vitellogenin only when treated with estrogen. After intensive studies on the protein chemistry and immunology of vitellogenin, we turned our attention to the regulatory phenomenon. Polysomal RNA from the liver of estrogen-treated roosters was translated in a reticulocyte cell-free system, and the newly synthesized protein identified by a highly specific and sensitive indirect immunoprecipitation reaction. The messenger-RNA that specifies vitellogenin has been purified more than 800-fold from rooster liver polysomal RNA by a combination of methods, including immunoprecipitation of polysomes and chromatography on poly(U)-Sepharose.

It is a pleasure to report that the process of moving the NUCLEIC ACID ENZYMOLOGY SECTION (Dr. M. Singer, Head) and installing it in the Laboratory of Biochemistry is essentially complete, and that work is progressing well. Developing relations with the Laboratory of Molecular Biology gives promise of stimulating and useful scientific communication with them.

The group working with Dr. Singer has continued with various aspects of the replication of viral DNA in monkey kidney cells infected with simian virus 40. Dr. Vogel has shown clearly that f1 histone, the lysine-rich histone, has a special affinity for superhelical DNA and that the extent of interaction depends directly on the degree of superhelicity. Dr. LeBlanc has examined the nature of the molecular species of viral DNA produced by several in vitro systems capable of synthesizing SV 40 DNA. Nuclear systems do not, by themselves, form complete circular duplex molecules, but addition of cytoplasm permits formation of circles. Addition of histones stimulates the formation of circular molecules. Studies on the nature of defective SV 40 DNA, substituted, by in vivo recombination, with host DNA sequences, is continuing. The system is very complex since with each passage of virus, changes in the structure and heterogeneity of the defective genomes occur. Dr. Rao, recently with the help of Dr. Blasi, is purifying and characterizing the defective species. Dr. Segal is in the process of determining the sequence of one portion of the host DNA that is incorporated into one of the defective species.

The group working with Dr. Klee (Drs. Barbahenn and Craine) have developed a fast purification procedure for polynucleotide phorsphorylase. The method is based on affinity chromatorgraphy with poly(U)-Sepharose and allows high yields of homogeneous, primer-independent enzyme. Structural and subunit studies on this enzyme, as well as the primer-dependent enzyme generated from it by limited proteolysis, are proceeding. Dr. Klee has developed a new assay procedure for cyclic-AMP phosphodiesterase. The method does not require the conversion of the product AMP to adenosine. The assay is being used for the purification of particulate and soluble phosphodiesterase. The mechanism of action of the enzyme and its regulation by interaction with activators and inhibitors will be investigated.

In the PROTEIN CHEMISTRY SECTION (Dr. E. Peterson, Head), Dr. McBride and his colleagues have continued their studies on the transfer of genetic information by incubation of recipient cells with chromosomes. They have identified as human in origin the hypoxanthine phosphoribosyltransferase gene products of four independent clones obtained by transforming mouse Aq cells with human metaphase chromosomes. However, human species of two other X-linked enzymes, glucose-6-phosphate dehydrogenase and phosphoglycerate kinase, were not found in any of these clones. Thus, only a portion of the X chromosome was expressed after transfer.

Of eleven clones obtained by selecting for the presence of thymidine kinase (TK) after transformation of TK-deficient B_{82} mouse cells with Chinese hamster chromosomes, two also contained the chromosomal donor species of galactokinase, indicating that closely linked genes are sometimes transferred together in this procedure. The clones were examined for the chromosomal donor species of nine nonlinked enzymes but none were detected, establishing that these clones had not arisen through cell fusion.

Dr. Evans and his co-workers have continued their studies of the biochemical changes accompanying the maturation of granulocytes in bone marrow. Using improved procedures for the isolation of subcellular fractions, they have found that the major glycoproteins of the cytoplasm of mature granulocytes (guinea pig) are associated mainly with the secondary granule fraction.

Their studies also show that the ornithine-utilizing enzymes, ornithine aminotransferase and ornithine decarbosylase, are present in immature cells but decline during the maturation of granulocytes in the bone marrow, whereas arginase, an ornithine-producing enzyme, increases with maturation.

Dr. Mage, seeking to develop methods for the specific isolation and study of cells involved in cellular immune reactions, has discovered that when antigen-reactive spleen cells are incubated on and briefly centrifuged on allogeneic target cell monolayers, some of the cells bearing graft vs. host activity are bound specifically. He is now attempting to ascertain which specific determinants must be present on the target cell monolayers for adsorption to occur.

Dr. Marciani has been investigating changes in the surfaces of chick embryo cells transformed by Rous sarcoma virus and by a temperature-sensitive mutant. He has discovered that transformation by either virus results in a significant decrease in two membrane-associated polypeptides having molecular weights of 250,000 and 73,000, respectively. Although shifting the temperature of cells infected with the mutant to the nonpermissive temperature (41°) caused a partial restoration of the larger polypeptide, along with the reversal of the morphological changes that had accompanied transformation, the inhibition of this restoration by substances that did not block the morphological changes makes it unlikely that this polypeptide has a primary role in the membrane transformation.

Dr. Marciani has also studied the binding of labeled concanavalin A to be the surfaces of these cells and has found that whereas normal cells and cells transformed by either virus at 37° showed the same relatively homogeneous binding behavior, cells infected by the mutant and maintained at 41° had at last two sets of receptors for concanavalin A. Such cells were agglutinable by concanavalin A only at the nonpermissive temperature (41°), and at this temperature they acquired a new property, the ability to adsorb red cells.

Z01 CBD 5210-07 LB
1. Laboratory of Biochemistry
2. Biochemistry of Gene Expression
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control of gene expression and growth in animal cells

Previous Serial No.: Same

Principal Investigator: E. Brad Thompson

Other Investigators: L. Lyons, T. V. Gopalakrishnan, A. Venetianer, D. Marciani, J. Pita, Y. Sakaue.

Cooperating Units: M.L. Lippman and D. Tormey, MODCT, NCI; S. S. Yang, LCB, NCI; R. Schwartz, Immunology Branch, NCI; J. Cheder and B. Wiggert, NICHD; G. Zubay and R. Owens, Columbia University; A. Tashjian and P. Dannies, Harvard Medical School; M. Paran, LTCB, NCI; A. Kandutsch and H. Chen, The Jackson Laboratory; L. Loriaux, RR, CH.

Man Years:

Total: 10
Professional: 6
Other: 4

Project Description:

Objectives: Understanding of the mechanism of gene regulation in eukaryotes, particularly as related to steroid hormone action and growth control. Application, where possible, of findings in these areas to clinical situations.

Methods Employed: These have been listed in previous reports. New techniques added include solid phase assay of steroid receptors and a method for enucleating a wide spectrum of cell types.

Major Findings:
1) Growth Control in cell hybrids. These studies (by Dr. Lyons) are nearing completion and are being prepared for publication. Three main results have been obtained:

a)1S + 1S hybrids between HTC (rat hepatoma) and L cells (transformed mouse fibroblast) show density dependent inhibition of growth although the parents do not. As chromosomes are shed, the hybrids return to the unregulated growth characteristic of their parents.

b) These hybrids, when injected into nude mice, show a striking delay in making tumors compared to their parental lines. The tumors from 1S + 1S hybrid cells were found to have reduced numbers of chromosomes.

c) The plasma membranes of the hybrids contain a protein present in neither parent, one which is similar in size to a marker protein for normal fibroblasts (Marciani).

2) The role of glucocorticoid receptors in gene control. Last year we reported hybridization experiments suggesting that the glucocorticoid receptor in HTC cells differed from that in L cells. New hybridizations have supported this view (Venetianer).

The import of this result is that the cell inhibitory responses of steroids in certain cells may be mediated by special steroid receptors.

Studies on enucleated cells have also continued (Gopalakrishnan) and a new method devised extending the cell types to which studies can be applied (MS submitted). We still aim to use enucleated cells in hybrids.

We have further characterized our series of non-inducible HTC cell variants. They are R^+, take up steroid, and concentrate it in the nucleus. Thus they appear blocked very late in their induction response to the hormone (Thompson).

Cell variants resistant to chloramphenicol and oubain have been developed (Gopalakrishnan) for use in hybridization.

3) Steroid receptors in human malignancies. Over 100 patients with breast cancer have been examined for receptors for several steroids. This is an effort to improve one's ability to predict those patients responsive to hormonal therapy (Tormey, Lippman). In addition to estrogen receptors, we have found androgen and progesterone receptors in certain patients, suggesting that these steroids also have a role in the biology of the disease.

4) Transforming virus in HTC cells. By appropriate cocultivation, a virus capable of assisting transformation in test cells can be evoked from HTC cells (Yang).

5) Control of cholesterol synthesis and of cell growth by oxygenated cholesterol derivatives. This collaboration (Kandutsch, Chen) has begun with 3 goals sought: to see whether cell receptors exist for cholesterol and for its derivatives; to explore cholesterol regulation in certain cell hybrids; and to seek cell variants in the cholesterol response pathways.

6) Completed and published or in press:
Serum-free line of HTC cells.
Ontogeny of steroid receptors in chick neural retina.
Steroid receptors in acute myelogenous leukemia.

7) New undertakings:

Purification of glucocorticoid receptors (Sakaue).
Search for cell-free transcription events in L cells (Zubay, Owens).
Study of control of steroidal and TRH-mediated induction events in GH3 x L cell hybrids (Tashjian, Dannies).
Attempt to see whether viral rescue by steroids is mediated by the standard steroid receptor mechanism (Paran).
Studies of drug interactions with steroid receptors (Pita, Loriaux).

Significance to Cancer Research: We continue to be concerned how animal cells' genes are regulated because we hope that knowledge in this area will be important to controlling cancer. Control of growth and cell responses to steroids seem good ways to approach this problem. We also continue to try to apply our results to clinical situations. The studies begun on breast cancer patients, derived directly from our basic research interest, will eventually provide data about the biology of the disease and assist in therapeutic decisions as well.

Proposed Course of Research:

1) Two more experiments are planned on the growth-controlled hybrids:
   a) Back-hybridize a clone of hybrid cells with its parent.
   b) Examination of cyclic nucleotides in these hybrids.

2) Continued development of hybrid cells and variant cell lines to study steroid action and gene control.

3) Steroid receptors in human malignancies:
   a) Breast cancer. Plan to initiate a prospective study on carefully stratified, matched cases to determine the value of androgen, progestational and glucocorticoid hormones in predicting therapy. Plan to collate clinical/biochemical data on cases already studied, for publication.
   b) Leukemia/lymphomas. New studies on glucocorticoid receptors and steroid action in these diseases are just being initiated.

4) Other projects mentioned will be continued.

Publications:

1. Thompson, E. Brad: Attachment of specific ligands and methods for specific proteins. Pyridoxamine phosphate. In Jakoby, W.B. and Wilchek, M. (Eds.): Methods in Enzymology, Vol. XXXIV. Academic Press, 1974, pp. 294-300.

2. Lippman, Marc and Thompson, E. Brad: The role of transcortin in glucocorticoid mediated enzyme induction: tyrosine aminotransferase induction in hepatoma tissue culture cells. J. Steroid Biochem. 5: 461-465, 1974.

3. Thompson, E. Brad, Lippman, Marc E. and Lyons, Linda B.: Control of enzyme induction and growth in tumor cells and cell hybrids. In Nakahara, W., Ono, T., Sugimura, T. and Sugana, H. (Eds.): Differentiation and Control of Malignancy of Tumor Cells. Tokyo, Japan, Univ. Tokyo Press, 1974, pp. 343-358.

4. Lippman, Marc E., Wiggert, Barbara O., Chader, Gerald J. and Thompson, E. Brad: Glucocorticoid receptors: characteristics, specificity, and ontogenesis in the embryonic chick neural retina. J. Biol. Chem, 249, No. 18: 5916-5917, 1974.

5. Lippman, Marc E., Perry, Seymour and Thompson, E. Brad: Cytoplasmic glucocorticoid-binding proteins in glucocorticoid-unresponsive human and mouse leukemic cell lines. Cancer Research 34: 1572-1576, 1974.

6. Lippman, Marc E. and Thompson, E. Brad: Steroid receptors and the mechanism of the specificity of glucocorticoid responsiveness of somatic cell hybrids between hepatoma tissue culture cells and mouse fibroblasts. J. Biol. Chem. 249, No. 8: 2483-2488, 1974.

7. Thompson, E. Brad: Gene expression in animal cells. In Anfinsen, C.B. and Schecter, A.N. (Eds.): Current Topics in Biochemistry. New York, Academic Press, 1974, pp. 187-217.

8. Thompson, E. Brad: Enzyme expression and growth control in HTC cells and hybrids of HTC cells with other cell types. In Davidson, Richard L. and de la Cruz, Felix (Eds): Somatic Cell Hybridization. New York, Raven Press, 1974, pp. 179-184.

9. Thompson, E. Brad, Anderson, C.U. and Lippman, M.E.: Serum-free growth of HTC cells containing glucocorticoid and insulin-inducible tyrosine aminotransferase and cytoplasmic glucocorticoid receptors. J. Cell Physiol. In Press.

10. Lippman, Marc E., Perry, Seymour and Thompson, E. Brad: Glucocorticoid-binding proteins in myeloblasts of acute myelogenous leukemia. In press in The American Journal of Medicine.

Keyword Descriptors: steroid/ cell hybridization/ enzyme induction/ growth control/ genetics/ oncogenic viruses/ gene control/ receptors.

Z01 CBD 366-05 LB
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biosynthesis and assembly of intracellular structural components

Previous Serial No.: Same

Principal Investigator: Kira K. Lueders

Other Investigators: Edward L. Kuff, Shoshona Segal, Dante Marciani, Samuel H. Wilson, Jan M. Orenstein

Cooperating Units: John E. Dahlberg, NCI

Man Years: Total: 4
Professional: 3
Other: 1

Project Description:

Objectives: To study the synthesis and transport of individual macromolecules and their assembly into complex intracellular components; to study the process of neoplastic transformation in terms of these coordinated functions.

Methods Employed: Cell fractionation by differential and density gradient centrifugation, isolated and gradient fractionation of nucleic acids and nucleoproteins, electron microscopy of isolated nucleoproteins, in vivo and in vitro incorporation of isotopically-labelled precursors into protein and nucleic acid, chromatographic, immunochemical and electrophoretic characterization of specific proteins, tissue culture of normal and transformed cells, nucleic acid hybridization, immunofluorescent staining.

Results: Studies during the past year were concerned primarily with intracisternal A-particles.

1. Studies on the biosynthesis and turnover of the A-particle main structural protein (P73) in cultured neuroblastoma cells were continued. The A-particle protein was found to turn over more rapidly than the average cell protein. The apparent half-life of A-particle protein relative to general cell protein was determined to be about 24 hours in the N4 neuroblastoma cell line and 30 hours in the NLIF hybrid line. It was calculated that on an absolute basis 60% of the label in A-particles is lost over a period of 24 hours. No extracellular A-particle protein was found although secretion contributed to turnover of general cell protein which was found to be 17% in each cell generation.

2. In collaboration with Drs. S. Wilson and J. Orenstein, the expression of A-particle markers was examined in cells induced by treatment with IUdR+dexamethasone to synthesis of C-type virus particles. The induction of C-type particles was demonstrated by electron microscopy as well as by characterization of the DNA-polymerase activity associated with the particulate fraction from the medium. Because the reaction properties of the DNA-polymerase associated with C-particles and A-particles are different, it was possible to assess the total amounts of the DNA-polymerase activities of the two particles in cells and in the culture fluid pellets. While the C-particle polymerase activity in the culture fluid increased 29-fold after treatment, the amount of A-particle DNA-polymerase activity did not change. Similarly, while C-particle group specific antigen levels were elevated 10-20 fold, there was no increase in A-particle group specific antigen. The proportion of synthesis of P73 as assessed by pulse labeling of cells and analysis of the A-particle antigen containing fraction on gels did not change when cells were induced to synthesize C-type virus. These experiments provide further evidence for independent control of expression of the genomes for these two types of particles.

3. RNA isolated from A-particles derived from myeloma was further studied by Dr. Shoshona Segal. The poly (rA)-containing RNA was found to stimulate amino acid incorporation by the cell-free system from wheat germ embryo. Examination of the protein products coded by this RNA revealed that a 30,000 molecular weight protein was the major product. When a similar RNA fraction prepared from total myeloma microsomes was used, this protein was not a major product suggesting that A-particle RNA does not represent a random collection of cellular messenger RNAs.

4. The possibility of repetitive sequences in the A-particle structural proteins of different molecular weights (73,000, 46,000, and 30,000) was further investigated and confirmed. The terminal amino group of the three different proteins was identified as arginine. The major (P73) protein revealed approximately half the number of peptides expected from the amino acid composition and the same number and similar pattern of peptides was found for the 46,000-dalton protein. The number of tryptophan residues in the 73,000-dalton protein was also half of that expected from the amino acid composition and was the same as that found in the 46,000-dalton protein. The repeating sequences appear to be connected linearly through conventional peptide bonds.

5. An attempt is being made to localize the intracellular site of synthesis of the particle protein. Cells were fractionated after a short pulse with an amino acid of high specific activity and A-particles and free- and membrane bound polyribosomes isolated. No evidence for a 73,000 -dalton protein has been found on polyribosomes. Attempts to label a 73,000-dalton protein in a cell-free system with preisolated polyribosomes have also been unsuccessful. A competition assay combined with immunoprecipitation has been used to demonstrate that the primary site of synthesis of A-particle protein is the bound polyribosomes. The major product specifically immunoprecipitated from the cell-free system by anti-A-particle serum is a 46,000 dalton protein. Minor products with molecular weights in the range 28-34,000-daltons are also present. It is possible that P73 is synthesized as a smaller unit with

post-translational processing of the protein shortly before or during assembly on the membrane.

In collaboration with Dr. J. Dahlberg, guinea pig virus preparations derived from tissue culture cells have been analyzed by gel electrophoresis in an attempt to characterize their intrinsic components. Protein patterns have confirmed that these particles resemble mouse mammary tumor virus rather than C-type virus in their structural proteins as had been suggested by their appearance by electron microscopy, the properties of their reverse transcriptase, and behavior in density gradients.

Proposed course of Research: Experiments will be designed to determine whether two specific A-particle properties such as P73 and A-particle DNA-polymerase are coordinately expressed in particles under different conditions: 1) in different phases of growth, 2) through the cell cycle of synchronized cells, 3) among different tissue culture lines.

Publications:

1. Marciani, D., and Kuff, E.L.: Structural Proteins of Intracisternal A-particles: Possible Repetitive Sequences. J. Virol 14: 1597-1955 (1974).

2. Teresky, A.K., Marsden, M., Kuff, E.L., and Levine, A.J.: Morphological Criteria for the in vitro differentiation of Embryoid Bodies produced by a transplantable Teratoma of Mice. J. Cell Physiol 84: 319-332 (1974).

3. Lueders, K.K., and Kuff, E.L.: Synthesis and turnover of Intracisternal A-particle Structural protein in cultured Neuroblastoma Cells. J. Biol Chem May 10, 1975.

Keyword Descriptors: Intracisternal A-Particles: characterization of structural proteins and RNA, synthesis and turnover.

Z01 CBD 379-14 LB
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural characterization of immunoglobulins, enzymes and other biomacromolecules.

Previous Serial No.: Same

Principal Investigator: Emma Shelton

Other Investigators: J. M. Orenstein

Cooperating Units: R. A. Lazzarini, Lab. Mol. Biol. NINDS
R. L. Switzer, Dept. Biochem, Univ. of Illinois, Urbana, Ill.

Man Years: Total: 4
Professional: 3
Other: 1

Project Description:

Objectives: To relate the fine structure of macromolecules to their biochemical and physical properties, and to their function in biological systems.

Methods and Major Findings:

1. Structure of Vesicular Stomatitis Virus (VSV)- (Orenstein & Lazzarini). A definitive study of the shape of VSV has shown that VSV buds from the cell membrane as a bacilliform unit, a shape that is similar to that of plant rhabdoviruses. Previously observed differences in shape are probably due to differences in membrane constituents derived from host cells. The "bullet" shape ascribed to VSV for years has been proved to be erroneous.

It has been shown by electron microscopy of thin sections of cells that ribosomes are closely associated with VSV as it buds from the cell membrane. This is taken as evidence that the RNP genome not only codes for itself, but also makes messenger RNA that codes for other viral components.

2. Structure of S. typhimurium phosphoribosylpyrophosphate synthetase (Switzer). We have completed the study of this enzyme and conclude that it is an asymmetrical pentamer.

Significance to Cancer Research: A knowledge of the structure of macromolecules obtained by observations with the electron microscope provides in many cases an unequivocal basis for knowledge of their function and similar confirmation of physical-chemical data. Thus we have evidence that the RNA of vesicular stomatitis virus can be transcribed without removal of the associated protein.

Proposed Course of Research: To elucidate further the structure of macromolecules and correlate their structure with their function.

Publications:

1. Roth, D.G., Shelton, E., and Deuel, T.F.: Purification and properties of phosphoribosyl pyrophosphate synthetase from rat liver. J. Biol. Chem. 249: 291-296, 1974.

2. Miller, R.E., Shelton, E., and Stadtman, E.R.: Zinc-induced paracrystalline aggregation of glutamine synthetase. Arch. Biochem. Biophys. 163: 155-177, 1974.

3. Shichi, H., and Shelton, E.: Assessment of physiological integrity of sonicated rod membranes. J. Supramolocular Structure 2: 7-16, 1974.

Keyword Descriptors: electron microscopy/ macromolecular structure.

Z01 CBD 945-02 LB
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Study of the Factors Regulating the Synthesis of Collagen during Embryonic Development and Growth of Cultured Fibroblasts

Previous Serial Number: Same

Principal Investigator: Beverly Peterkofsky

Other Investigators: Ryuichiro Hata, M.D., Visiting Fellow, Carlotta Evans, D.D.S.

Cooperating Units: None

Man Years: Total: 4.7
Professional: 2.7
Other: 2.0

Project Description:

Objectives: To elucidate the basic mechanisms of collagen synthesis and secretion and to investigate the factors which regulate these processes in cultured fibroblasts, both normal and transformed, and during embryonic development.

Methods Employed: Proteins of whole chick embryos, isolated bones of these embryos, or cell-free systems derived from these bones or cultured fibroblasts are labeled with ^{14}C-proline. The proteins are precipitated with trichloroacetic acid, redissolved and then assayed for radioactivity in collagen and noncollagenous proteins by a method involving specific digestion of collagen in a mixture of proteins using highly purified collagenase. This method was developed in our laboratory and is described in a previous report (1968-9). The relative rate of collagen synthesis can be calculated from data obtained by this method and the specific rate of synthesis in tissue or cells is calculated by determining the DNA content and expressing the rate as cpm/μg DNA. The proteins of the cell and medium fractions of cultured fibroblasts are analyzed separately in order to study secretion. Morphological changes in cultured cells are recorded by polaroid photomicroscopy.

Cell-free incorporation of ^{14}-C-proline is studied with a system consisting of membrane-bound or free polysomes derived from chick embryo bone tissue and soluble factors derived from chick embryo liver, a tissue which synthesizes very little collagen, so that variations in collagen synthesizing polysomes of a tissue may be studied independently of possible variations in the soluble fraction.

To determine the level of proline hydroxylation in collagen of cells or tissues labeled with radioactive proline, collagenase digests are hydrolyzed with 6N HCl and radioactive proline and hydroxyproline in the hydrolyzates are measured by a specific radioassay. To measure the level of lysine hydroxylation, cells or tissues are labeled with radioactive lysine and hydrolyzed collagenase digests are chromatographed on Dowex-50 in 2N HCl, which separates lysine and hydroxylysine.

Prolyl hydroxylase is measured by tritium release from H^3-proline labeled unhydroxylated collagen prepared by incubating chick embryo frontal bones with H^3-proline in the presence of the iron chelator αα-dipyridyl, which inhibits hydroxylation.

Cell lines used in these studies: Chick embryo fibroblasts prepared by digesting frontal bones from 15 day chick embryos with crude collagenase and culturing the released cells. Subcultures in the second to fifth passage are used.

L-929- a line of mouse embryo fibroblasts established about 30 years ago by use of a chemical carcinogen.

Balb-3T3- a contact inhibited line of mouse embryo fibroblasts isolated by Todaro and Aaronson.

Balb-3T6 and 3T12- lines of mouse embryo fibroblasts derived from the same pool of cells as 3T3 but non-contact inhibited and tumorigenic.

Ki-3T3- a line established by Aaronson by transformation with Kirsten sarcoma virus. These cells do not produce viral particles unless super-infected with murine leukemia virus.

Embryonic and adult human diploid fibroblasts-purchased commercially.

Major Findings:

I. Quantitation and localization of prolyl hydroxylase.

Because of the relationship between hydroxylation of proline residues in collagen and collagen secretion, we have been studying the localization of prolyl hydroxylase. We have found that at least 80% of the prolyl hydroxylase in homogenates of chick embryo limb bones is located in vesicles of the endoplasmic reticulum (microsomes). The site of synthesis of the collagen polypeptide has also been localized in these particles in our previous studies. By studying the effects of varying concentrations of two different nonionic detergents, Triton X-100 and Brij-35, on these vesicles, we have determined that 85-90% of the particulate prolyl hydroxylase is localized within the cisternae. This conclusion is based on the observation that very low concentrations of these detergents which do not disrupt the general structure of the particle will allow the solubilization of 85-90% of the hydroxylase activity.

Trypsin, and other large protein molecules, cannot enter microsomal cisternae, yet trypsin treatment of the particles results in inactivation of the small fraction of enzyme which is active in the absence of detergent, suggesting that 10-15% of the hydroxylase activity is present at or near the surface of the particle. The enzyme which is active only in the presence of detergent is almost unaffected by trypsin treatment, even at relatively high concentrations of trypsin, further confirming the intracisternal nature of the majority of the prolyl hydroxylase activity.

II. Regulation of collagen synthesis and secretion by ascorbic acid.
A. Chick embryo fibroblasts. The characteristics of collagen secreted into the medium of chick embryo fibroblasts cultured with ascorbate, α,α'-dipyridyl or with no additions has been investigated using gel filtration on an agarose column. It was found that under conditions which led to synthesis of underhydroxylated collagen (no additions or plus α,α'-dipyridyl), a significant portion of the collagenous protein in the medium was smaller than procollagen chains and probably represents degraded material. These results suggest that underhydroxylated collagen is susceptible to degradative enzymes in the cell cultures.

B. Transformed Ki-3T3 fibroblasts. The observations that proline in collagen synthesized by transformed 3T3 cells was almost completely hydroxylated in the absence of exogenous ascorbate was further investigated. Our studies show that ascorbate does not accumulate in these cultures nor do homogenates of these cells synthesize ^{14}C-ascorbate or earlier intermediates from ^{14}C-glucuronic acid. However, when a 20,000 x g supernatant fraction from a crude cell sonicate was added to an assay system for prolyl hydroxylase, the supernatant partially replaced ascorbate as reducing agent. This further supports the conclusion that transformed 3T3 cells synthesize a compound which can function as a reducing agent in the prolyl hydroxylase reaction.

III. The effects of dibutyryl cyclic AMP (dbcAMP) on transformed Ki-3T3 fibroblasts.
A. The effect of dbcAMP on proline transport was investigated since it had been observed in our previous studies that in addition to the 5-8 fold stimulation of ^{14}C-proline incorporation into collagen there was a 1.5-3 fold stimulation of incorporation into noncollagenous protein. This increase could be accounted for either by an increase in the synthesis of several specific cellular proteins or by an increase in ^{14}C-proline transport which would result in increased labeling of all cellular proteins. The amount of free ^{14}C-proline in the amino acid pool (acid soluble) of Ki-3T3 cells grown in the presence or absence of dbcAMP was measured as well as incorporation into collagen and noncollagen protein. The increase of ^{14}C-proline in the free pool of dbcAMP treated cells (1.5 fold) almost totally accounted for the increased incorporation into noncollagen protein (1.8 fold) but not into collagen 4.7 fold). Proline transport was also measured in the presence of cycloheximide, an inhibitor of protein synthesis, in order to measure the transport reaction specifically. It was found that dbcAMP treatment increased proline transport 1.5-2 fold and that the apparent K_m was increased. Since transformed cells have been found to exhibit a faster rate of transport for several amino acids compared to the parent cells, it might be expected that dbcAMP, which causes reversal of several transformed properties, would decrease the rate of proline transport. Furthermore, the increased amino acid transport in transformed cells

is associated with a change in V_{max} rather than K_m, suggesting that the observed increase in proline transport is not an effect specifically related to cAMP metabolism. This conclusion was supported by the finding that growth of Ki-3T3 cells in dbcGMP or butyrate also increased proline transport.

B. The effect of dbcAMP on prolyl hydroxylase has been determined since it has been proposed by others that this enzyme plays a rate regulating role in collagen synthesis. Growth of transformed fibroblasts in dbcAMP did not affect the levels of prolyl hydroxylase activity under conditions in which the relative rate of collagen synthesis was increased.

Proposed Course of Research

I. Prolyl Hydroxylase location. Experiments will be carried out a) to confirm the existence of two locations for prolyl-hydroxylase in endoplasmic reticulum vesicles and b) to determine whether the enzymes in both locations function under normal physiological conditions and the significance of two sites in relation to collagen synthesis and secretion.

II. Effect of ascorbate on collagen synthesis and secretion.
A. It will be determined whether degradative products of underhydroxylated collagen, which we have observed in the medium of chick embryo fibroblasts, are formed intra or extra-cellularly and whether such degradation plays a role in regulating the total amount of collagen synthesized.

B. Further experiments will be carried out to isolate large amounts of the reducing agent in transformed cells for purposes of identification and characterization.

III. Effect of dbcAMP on transformed fibroblasts. The properties of collagen synthesized at increased rates in dbcAMP grown transformed fibroblasts will be studied to determine whether there is a switch in the genetic type of collagen. This will be accomplished by characterizing chains of denatured collagen on DEAE and CM-cellulose columns.

Significance to Biomedical Research and the Program of the Institute. Determining the precise mechanism by which vitamin C affects collagen synthesis should lead to a more accurate use of this vitamin. In addition, our studies with transformed cells indicate that, at least under some conditions, animal cells may be able to synthesize a substitute for the function of vitamin C in connective tissue.

Our studies with dbcAMP are aimed at determining the extent to which this chemical reverses cell transformation.

Publications:

1. Peterkofsky, B., and Prather, W.B.: Increased collagen synthesis in Kirsten Sarcoma Virus-transformed Balb 3T3 cells grown in the presence of dibutyl cAMP Cell, 3, 291-299 (1974).

2. Peterkofsky, B., and DiBlasio, R.: Modification of the tritium-release assays for prolyl and lysyl hydroxylases using Dowex-50 columns. Anal. Biochem. 61 In Press (1975).

Keyword Descriptors: regulation/ collagen biosynthesis and secretion/ ascorbate/ dibutyryl cyclic AMP/ proline hydroxylation.

Z01 CBD 5214-04 CB
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: DNA synthesis in mammalian cells

Previous Serial No.: Same

Principal Investigator: Samuel H. Wilson

Other Investigators: A. Matsukage, J.D. Minna, T. Marshall, E.L. Kuff, J. Orenstein

Cooperating Units: National Heart and Lung Institute

Man Years: Total: 3
Professional: 2
Other: 1

Project Description:

Objective: The broad objective of this research program is to understand mechanisms involved in DNA synthesis in mammalian cells. Our approach in the initial phase has been investigation of DNA polymerases in the mouse plasmacytoma, MOPC-104E; eventually we hope to use purified MOPC-104E DNA polymerase in the development of an in vitro system capable of replicating mammalian DNA.

Our previous analysis of the DNA polymerase content of MOPC-104E revealed five distinct forms of enzyme that possessed relatively abundant activity. Two of these enzymes appeared analogous to DNA polymerases well known in other mammalian tissues, and a third enzyme exhibited properties similar to those of the mouse type-C virus-associated DNA polymerases. The other enzymes had not been clearly recognized previously. During the past two years other laboratories have also detected these new enzymes, and areas of investigation concerning their properties and biological roles have emerged.

One of the most important questions to be answered concerning the mammalian DNA polymerases may be stated as follows: What is the molecular relationship, if any, between the various forms of DNA polymerase that appear to be present in each cell: Currently, the subunit structure of only one of the main cellular DNA polymerases is known. We anticipate that our studies on the properties of the purified myeloma DNA polymerases will help answer this important question, and provide a sound basis for future work on mechanisms of DNA synthesis using in vitro systems.

Methods Employed: Procedures were developed for the purification of DNA polymerases in mouse tissue. The procedures involved subcellular fractionation, various types of column chromatography and assays for DNA polymerase activities using a variety of reaction conditions. Cell fractionation procedures and characterizations of reaction products were performed using conventional techniques of rate-zonal centrifugation and scintillation spectrophotometry.

Major Findings:
1. Resolution of DNA polymerase I from mouse myeloma into three separate molecular forms: We found that a preparation of the high molecular weight DNA-directed DNA polymerase, which had been designated DNA polymerase I, actually contained a mixture of several molecular species. The molecular species differed in molecular weight, and were also found to differ in isoelectric points. Thus, the original mixture of species was easily resolved using serial steps of ion exchange column chromatography. One of the newly resolved species appeared to be identical to the enzyme that had been previously designated DNA polymerase IV; it is now designated DNA polymerase IA, whereas the other two newly resolved species are now designated DNA polymerase IB and IC, respectively. So far as tested, the catalytic activities of these three forms of enzyme have been very similar; the activity of each is more than 20-fold higher in myeloma than in adult mouse liver, and can be recovered from purified myeloma nuclei as well as the cytoplasmic S-100 fraction.

The various DNA polymerases detected thus far in our studies may be summarized as follows:

Summary of the Main Forms of MOPC-104E DNA Polymerase

Designation of DNA Polymerase	Some Distinguishing Features
DNA polymerase II	25,000 M.W.; pI of 9.4.
DNA polymerase III	About 250,000 M.W.; Tentative subunit composition of 72,000, 67,000 and 50,000; both RNA and DNA-directed.
DNA polymerase IA (or IV)	DNA-directed only; higher molecular weight and isoelectric point than IB and IC.
DNA polymerase IB	DNA-directed only; the main form of DNA polymerase I.
DNA polymerase IC	DNA-directed only; lower molecular weight and isoelectric point than IA and IC.
6S DNA polymerase	RNA-directed only.

These forms of enzyme may be separated from each other on the basis of their distinct physical properties; we believe that none are mitochondrial or viral in origin.

2. Studies on the subunit structure of the mouse myeloma DNA polymerases: The amounts of DNA polymerase III protein obtained during our previous studies were insufficient to permit analysis of the subunit composition of the enzyme. During the past year, we have purified quantities of DNA polymerase III and of DNA polymerases IA, IB and IC sufficient for determination of subunit composition. Analysis of these enzyme preparations by polyacrylamide gel electrophoresis under non-dissociating conditions revealed that each enzyme preparation contained only one detectable Coomassie blue stainable, protein band; essentially all of the DNA polymerase activity detected in these gels co-migrated with the protein band. Experiments to define the subunit composition of the various polymerases are now under way, and the initial results indicate that DNA polymerase III contains subunits of apparent molecular weights of about 72,000, 62,000 and 50,000.

3. Use of RNA primers and DNA templates by DNA Polymerases IA, IB and IC: DNA polymerases IA, IB and IC not active with single stranded calf thymus DNA as template, but are very active when short DNA primers are hybridized to the single stranded DNA. Since some types of in vivo DNA synthesis may involve addition of deoxynucleotides onto the ends of short RNA primers, we examined the effect of RNA primers on the ability of the polymerases to use various DNAs as template. After RNA primers were provided by the action of *E. coli* RNA polymerase, single stranded calf thymus DNA was copied. It was also copied in the presence of the short RNA primer, oligo(rA)$_{10}$. These results suggested that calf thymus DNA contained a region of poly(dT), and that the enzymes were capable of initiating DNA synthesis on a poly(dT) oligo(rA) template-primer combination. A systematic study on the use of various combinations of synthetic templates and primers revealed that poly (dT) oligo(rA) was by far the most effective for promoting DNA synthesis. This preference of DNA polymerase I for poly(dT) oligo(rA) might reflect its in vivo specificity for initiation of DNA synthesis.

4. Type-C Particle Expression in human x mouse hybrid cell segregating human chromosomes: This work was performed in collaboration with Dr. J.D. Minna and co-workers (NHLI). In order to investigate possible effects of human genes upon type-C particle metabolism, freshly derived human cells were fused with mouse cells that were producing large numbers of type-C particles. As the fused cells were grown in culture, human chromosomes were preferentially lost. During this period of segregation of human chromosomes, the metabolism of mouse type-C particles was examined. The results suggest that human genes can alter the amounts of several mouse type-C particle components that are both present in the cell and released into the medium. One such type-C particle component, the DNA polymerase activity, was investigated in detail. Regulation of the packaging and release of the polymerase activity within C-particles may be attributed to genes located on human chromosomes 12, 14 and 21.

5. More Evidence that Mouse Intracisternal A-Particles and C-Particles bear no direct precursor-product relationship: Our continuing studies on the metabolism of intracisternal A-particles and C-particles have revealed an example of "non-identical regulation" of the metabolism of the two kinds of particles. Treatment of mouse neuroblastoma N-4 cells with a combination of IUdR and dexamethasone resulted in no detectable change in the metabolism of A-particles; in contrast, C-particle metabolism was changed markedly. Aspects of this work are described in Annual Reports No.___ prepared by Dr. E.L. Kuff. It should be noted that the change in C-particle metabolism after IUdR and dexamethasone treatment was first described by R.C. Gallo and co-workers (NCI).

Significance to Cancer Research: Understanding in detail of the mechanisms involved in DNA synthesis is vital to our understanding the molecular biology of neoplasia. Many approaches are being used to investigate DNA synthesis in normal and neoplastic cells, and advances in our understanding on all levels will prove useful in preventing, treating, and ultimately controlling cancer. Activity of DNA synthesizing enzymes is necessary for maintenance of a rapid rate of cell division, and in some cases these enzymes play an important role in the development of the neoplastic state.

Proposed Course of Research:

1. To determine the DNA polymerase content of murine tissues in various stages of growth regulation and polyoma virus infection, using methods of systematic fractionation and detection of the DNA polymerases.

2. To further purify and characterize the forms of MOPC-104E DNA polymerase.

3. To investigate the replication of well-defined DNAs using an *in vitro* system consisting of purified components.

4. To continue investigations of oncornavirus and DNA polymerase expression in human x mouse hybrids.

5. To purify and characterize the intracisternal A-particle-associated DNA polymerase.

Publications:

1. Stromberg, K., and Wilson, S.H.: Structural Studies of Avian Myeloblastosis Virus. Selective Release of Ribonucleoprotein polypeptides from the core component and partial purification of the DNA polymerase. Biochim. Biophys. Acta, 361: (1974) 53-58.

2. Schrier, B.K., and Wilson, S.H.: On the Measurement of Tritium in DNA and its Application to Assay of DNA Polymerase. In Prescott, D.M. (Ed.) Methods In Cell Biology. New York, Academic Press, Inc. 1975. In Press.

3. Matsukage, A., Bohn, E.W., Wilson, S.H.: On the DNA polymerase III of mouse myeloma. Partial purification and characterization. Biochemistry, 14: 1006-1020 (1975).

4. Matsukage, A., Bohn, E.W., Wilson, S.H.: Differential sensitivity of the low molecular weight DNA polymerase to sulfhydryl blocking reagents. Biochim Biophys. Acta (1975) 383: 338-343.

5. Miller, Jr., J.V., Thompson, E.B., Kuff, E.L., and Wilson, S.H.: Polydeoxythymidylate inhibition of Rabbit Reticulocyte RNA dependent protein synthesis in a Knebs II oscites cell system. Biochem. Biophys. Res. Commun. (1972) 48: 1280-1286.

6. Miller, Jr. J.V., Wilson, S.H., Kuff, E.L., and Thompson, E.B.: Inhibition of cell-free globin synthesis by polydeoxythymidylate. Biochim. Biophys. Acta, 294 (1973) 507-516.

Keyword Descriptors: DNA replication and the DNA synthesizing enzymes in mammalian cells.

Z01 CBD 5215-04 LB
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biological and ultrastructural studies on cell cooperation in the immune response.

Previous Serial No.: Same

Principal Investigator: Emma Shelton

Other Investigators: Jan Orenstein

Man Years: Total: 3
Professional: 2
Other: 1

Project Description:

Objective: To examine the interaction of lymphocytes and macrophages cooperating in the immune response and specifically: to examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen.

Methods and Major Findings:

1. Growth of small numbers of cells in diffusion chambers. Comparatively small numbers of cells, 2.5 - 10 x 10^4, of primed mouse peritoneal cells cultured in diffusion chambers, can with antigen, be induced to differentiate into plasma cells producing antibody specific to two different priming antigens, horseradish peroxidase and sheep erythrocytes. This number of immunocytes is small enough to permit morphologic analysis of the events of differentiation. It was found that plasmablasts and mature plasma cells formed very prominent, long-term associations with macrophages. This association might explain the observed absence of plasma cells in blood and lymph *in vivo*. In many plasma cells responding to horseradish peroxidase, no specific antibody could be detected by immunohistochemical techniques. This phenomenon may have a bearing upon the synthesis and secretion of specific antibody by individual cells.

2. Scanning electron microscopy of mouse peritoneal cells. A study was undertaken to characterize the surface features of mouse peritoneal cells that had settled on Millipore filters or on glass in order to gain an understanding of how they interacted with artificial substrates and with each other. Lymphocytes, macrophages, and mast cells extend delicate projections, "direction-oriented processes", toward nearby cells. The processes arise from a roughly circular

region of the cell circumference and vary in length up to 1.5 μM. It is suggested that they are related to communication between these free cells that do not join together into a fixed tissue. The surface of the macrophage was profoundly altered by contact with the foreign environment but the lympocyte surface appeared to be unaffected.

Proposed Course: To examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen. And specifically: to continue with scanning electron microscopy to: characterize and compare surface morphology to lymphocytes derived from the peritoneal cavity, thymus and lymphnode. To describe surface morphology of cells that have differentiated into plasma cells in diffusion chambers.

To prepare hybrid antibody to tag T and B cells for identification in SEM and to follow fate of these cells in differentiation in diffusion chambers.

To continue with replica technique and to look at retention of HRP on macrophage surfaces.

Publications:

Shelton, E., and Orenstein, J. M.: Localized membrane changes in lymphocytes and macrophages interacting with each other and with antigen. Proc. Eighth Int. Congress on Elec. Micr. Vol. II. Sanders & Goodchild, (Eds.) pp. 244-245, 1974.

Keyword Descriptors: electron microscopy/ plasma cell differentiation/ lymphocytes/ macrophages/ membranes.

Z01 CBD 3212-01 LB
1. Laboratory of Biochemistry
2. Cell Physiology and Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on cell nutrition, growth and neoplastic transformation *in vitro*

Previous Serial No.: Same

Principal Investigator: F. M. Price, K. K. Sanford

Other Investigator: C. Fox, R. R. Gantt, R. F. Camalier, S. L. Handleman

Cooperating Units: R. Parshad, Department of Pathology, Howard University, Washington, D. C. Laboratory of Cell Biology, NCI; K. Kohn, Laboratory of Molecular Pharmacology, NCI

Man Years:
Total: 1 5/8
Professional: 1/8
Other: 1 ½

Project Description: A program to improve culture conditions so that rigidly controlled cells can be used to study differences between nonneoplastic and neoplastic cells at nutritional, metabolic, karyologic and morphologic levels.

Objectives:

1. To determine the influence of peptone on the growth and neoplastic transformation of cell lines derived from minced C3H mouse embryos.

2. To study the effect of serum fractions on the growth and neoplastic transformation of C3H mouse embryo cell lines.

3. To determine the effect of horse and fetal bovine serum and 1% and 18% oxygen on chromosome stability, presence of fetal antigen and DNA breakage of C57 mouse lung cells.

4. To develop techniques for culturing normal human epithelial cells and to attempt to transform these cells by chemical carcinogens and study differences between normal and neoplastic human epithelial cells at nutritional, metabolic, karyologic and morphologic levels.

Methods Employed: Freshly explanted mouse cells were grown with Witte's peptone used as a supplement to chemically defined medium to determine its effect on growth and neoplastic transformation of these cells. C3H mouse cells were also grown with large and small molecule fractions of mare and stallion serum to determine the effects of these fractions on growth and neoplastic transformation.

Cultures derived from adult C57 mouse lung were cultured in medium containing either horse serum or fetal bovine serum and cultures grown in both sera were gassed with 1% and 18% oxygen-containing gas. The cells were tested at various intervals for presence of fetal antigen, changes in chromosomes and DNA breakage.

Techniques were devised to culture normal human epithelial cells. Primary cultures of human epithelial cells were treated with various chemical carcinogens to attempt to induce neoplastic transformation.

Major Findings: Growth of freshly explanted C34 mouse cells in medium supplemented with Witte peptone.

1. Two cell lines from C3H mouse embryos were initiated in chemically defined medium supplemented with 15% Witte peptone. The peptone supplement was about as effective as serum in supporting cell growth with the advantage of being autoclavable. After several months in vitro these cell lines underwent cytologic changes and neoplastic transformation as tested by implantation of cells in C3H mice. The peptone used in this initial study was several years old and no more of that lot was obtainable. Attempts to duplicate our first finding with presently obtainable Witte peptone resulted in very poor growth of the cells, and no cell lines could be established using the new peptone.

The effect of serum fractions on the growth and neoplastic transformation of C3H mouse embryo cells.

Three pools of C3H mouse embryo cells were grown in NCTC 135 supplemented with large and small molecule fractions of fetal bovine serum, stallion serum and mare serum. Best growth was obtained in medium containing the large molecule fraction of fetal bovine serum. In media containing both large and small molecule fractions of mare and stallion serum growth was variable. Cells cultured in medium containing only the large molecule fraction of mare or stallion serum with one exception could not be established as long-term lines. Cells grown in the various fractions were examined at 3 week intervals for cytologic changes and also were injected periodically into syngeneic hosts for detection of neoplastic transformation. Analysis of these results is in progress. The results to date show a correlation between tumor production and morphologic changes. Cells grown in each of the 3 media containing large molecule fetal bovine serum exhibited a markedly enhanced chromosome stability.

It does not appear that any fraction or combination of fractions produced reproducible neoplastic transformation in all 3 of the cell pools.

3. The effect of horse and fetal bovine serum and 1% and 18% oxygen on chromosome stability, DNA breakage and production of fetal antigen in C57 mouse lung cultures.

The chromosome number of C57 mouse lung cells remained more stable when the cells were grown in an atmosphere containing 1% oxygen than when the cells were cultured in the presence of 18% oxygen. This effect was noted regardless of the serum which was in the culture medium.

Tests are still being run to determine the effect of horse and fetal bovine serum and 1% and 18% oxygen on DNA breakage and repair of the lung cells.

All of the cell lines tested showed no presence of fetal antigen through 10 weeks in vitro. After approximately 20 weeks in culture the cells grown in fetal bovine serum and 1% oxygen showed some fetal antigen. After 40 weeks in vitro the cells in horse serum and 1% oxygen showed fetal antigen as well as those cells in fetal bovine serum in either oxygen concentrations. There appeared to be more fetal antigen present, however in the cells grown in fetal bovine serum in 1% oxygen.

4. Development of techniques for the cultivation of human epithelial cells and treatment of those cells with chemical carcinogens.

Initially we were starting human epithelial cultures by enzymatically separating epidermis from dermis. Several enzymes including trypsin, collagenase and viokase have been used for this separation. However, using these methods to initiate cultures was not satisfactory since the cells obtained have not grown. We are now initiating human epithelial cultures by using the explant method. First the flask is coated with horse serum by adding 2ml serum The serum coating allows good attachment of the explants and also may be of nutritive value. The skin to be used is washed 5 times in phosphate buffered saline and the underlying fat and as much dermis as is possible trimmed off The skin is then cut into pieces 1-2mm square. About 5-10 pieces of skin are placed in each flask, epidermis side up. The flasks are allowed to set upright in a 37.5°C incubator overnight to allow the explants to attachment. The following day 2-3ml medium NCTC135 plus 10% horse serum is added to the flask. In 7-10 days epithelial growth can be seen around the explants at which time the explants are removed to prevent growth of fibroblasts.

Primary human epithelial cultures obtained in this manner were treated with the chemical carcinogen Benzpyrene. Concentrations of 5, 10, and 50 μg benzpyrene have been found to be toxic and thus far we have been unable to note any morphologic changes which may be indicative of neoplastic transformation. Treatment with 14C labeled benzpyrene have shown that human skin epithelial cells do metabolize benzpyrene.

Significance and Proposed Course:

1. The use of a peptone as a protein supplement to chemically defined medium has an advantage over serum in that the peptone can be autoclaved thus eliminating possible contamination with viruses or mycoplasma. However, since the presently obtainable Witte peptone does not appear to replace serum as a protein supplement as indicated in our initial studies, this project has been dropped and no further use of Witte peptone will be made.

2. No serum fraction or combination of fraction appears to consistantly transform mouse embryo cells. However, cytologic diagnosis of transformation consistantly agrees with injection assays for neoplastic transformation. This strengthens the use of cytologic examinations to determine if cells are neoplastic or not.

It also appears that there may be differences between different embryo pools which may be responsible for inconsistant effects of the serum fractions. This could have important genetic implications.

A manuscript of this study is being prepared.

3. Data from this project is presently being analyzed. We hope to be able to correlate changes in chromosomes, DNA and fetal antigen with certain culture conditions and with neoplastic transformation. Thus it may be possible to culture cells, keeping them in a nonneoplastic state. This would be useful for carrying out studies with known carcinogenic agents.

4. Most human cancers are carcinomas. It would be beneficial if in vitro studies can be carried out with epithelial cells rather than fibroblasts. Traditionally however most in vitro cancer studies have used fibroblasts since they are much easier to cultivate. It is vitally necessary to develop techniques allowing investigators to culture and study human epithelial cells. We will continue to improve methods for the cultivation of these cells. We will use these cells to further study neoplastic transformation by carcinogenic agents.

Keyword Descriptors: cell nutrition/ serum fractions/ transformation/ epithelial cells/ chemical carcinogens.

Z01 CBD 3213-01 LB
1. Laboratory of Biochemistry
2. Cell Physiology and Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinogenesis in vitro and its manifestations

Previous Serial No.: Same

Principal Investigator: K. K. Sanford

Other Investigators: C. H. Fox, R. R. Gantt, S. L. Handleman, G. M. Jones,
F. M. Price

Cooperating Units: Masakazu Hatanaka, Flow Laboratories, Rockville, Md.
Bruce Wetzel, Dermatology Branch, NCI
Ram Parshad, Department of Pathology, Howard University
School of Medicine, Washington, D.C.,
Elli Kohen, Papanicolaou Cancer Research Institute,
Miami, Florida.

Man Years:
Total: 2 ¼
Professional: ½
Other: 1 3/4

Project Description:

Objectives:

I. To identify causative factors in "spontaneous" neoplastic transformation.

II. To identify any characteristics of cells in vitro that may be directly or indirectly associated with their neoplastic transforma

Methods Employed: General methods will be described under each study.

Major Findings:

I. Causative Factors

The "spontaneous" neoplastic transformation of cells in culture that occurs without the deliberate addition of viruses or carcinogens is probabl our best model of carcinogenesis as it occurs naturally in animals includin man. Although this phenomenon is referred to as "spontaneous", its occurrence is influenced by culture conditions and we believe there are extrinsi causes. We have considered two working hypotheses for transformation in mouse cells: 1) Under certain conditions DNA produced by RNA viruses of the murine leukemia complex may become integrated into host DNA at critical sites and produce mutations affecting growth control, 2) Alternatively, chromosomal breakages and rearrangements with possible alterations in DNA may be causative.

Study 1. - Search for Moloney sarcoma virus (MSV) RNA in spontaneously transformed mouse cell lines. (Collaboration with Dr. Hatanaka).

The objective of this study was to determine whether MSV-RNA could be detected in $C3H_f$ mouse cells by molecular hybridization of a DNA probe synthesized on MSV-RNA template by means of reverse transcriptase. Cell lines had undergone spontaneous neoplastic transformation as tested by in vivo assay and gave negative tests for gs leukemia virus antigens. No sarcoma virion was detected in these cell lines when hybridized with the DNA probe. These experiments provide the most sensitive assays to date for detecting virus expression, and the results are consistent with the postulate that "spontaneous" neoplastic transformation does not correlate with expression of MSV.

Study 2. - Effects of oxygen, zinc, serum and serum fractions on chromosome stability of cells in culture (Collaboration with Dr. Parshad).

Experimental studies confirm and extend previous observations that fetal bovine serum (FBS) as compared with horse serum (HS) promotes karyotypic stability in freshly isolated adult and embryonic mouse cells. The significantly higher zinc level in FBS is not the cause of this difference. In fractionation experiments, the large molecule fraction of HS as compared with all other fractions of HS or FBS is associated with an increased shift from diploidy and appearance of abnormal chromosomes. Mouse cell cultures grown in the presence of 1% oxygen (as compared with atmospheric 18%) show a diminished rate of both structural chromosomal alterations and shift from diploidy. Chromosome stability in the presence of a decreased oxygen concentration appears to be a general phenomenon in cultured mouse cells. Thus, both oxygen concentration and certain serum fractions have been identified as causative factors in chromosomal instability. In spite of the stabilizing influence of low O_2 and FBS on chromosomes of the mouse cells studied, mouse chromosomal alterations ultimately occurred and the cells underwent neoplastic transformation.

Proposed Course: Two studies will be continued: 1. Clones of Chinese hamster cells that underwent neoplastic transformation in vitro, grew as sarcomas in vivo, and were subsequently returned to culture, contain a high proportion of diploid cells. The clones were from a female and were implanted into males so the tumor cells can be distinguished from host stromal cells by the sex marker chromosomes. The chromosomes will be banded to see if any specific chromosomal alteration can be detected and be associated with spontaneous neoplastic transformation.

2. In collaboration with Dr. Kurt Kohn (Lab. of Molecular Pharmocology, NCI) we are examining the possibility that chromosomal alterations produced by O_2 and serum are manifestations of DNA damage. The elution pattern of thymidine-labeled DNA provides an estimate of DNA chain length in cells grown in HS and high O_2 as compared with those in FBS and low O_2. Some differences have been observed but we are currently assessing whether the damaged DNA results from trauma in removing cells from the growth surface by trypsin. A new method of growing cells for this technique is being developed.

In addition, we are placing emphasis on carcinogenesis of human cells (See Serial No. 5229) for several reasons including our primary interest in human cancer; the fact that human chromosomes are more stable; human cells appear to be free of endogenous viruses, and human cells can now be assayed in nude mice.

II. Manifestations of neoplastic transformation

The development and banking of frozen stocks of paired neoplastic and non-neoplastic cells of common origin has provided us with invaluable controlled biologic material for studying those cell properties that are correlated with neoplastic state. These paired lines, some from the same single cell, are being used in many of the studies in this Section.

Study 1. Cell surfaces during the mitotic cycle examined by scanning electron microscopy (SEM) and cinephotomicrography in a clone of non-neoplastic and derivative neoplastic cells. Cell surface features have been considered indices of neoplastic transformation in culture. Drastic changes in surface morphology also reportedly occur as cells profess through the mitotic cycle. (Collaboration with Dr. Wetzel) By combining cinephotomicrography with SEM the postmitotic time (hence approximate stage in the cell cycle) and surface morphology of the same individual cells could be determined under physiologic growth conditions. This approach was applied to 14 cultures of 6 non-neoplastic fibroblast lines and 19 cultures of 3 neoplastic lines of common single cell origin grown under the same culture conditions. No certain correlation could be established between surface morphology and the cell cycle in interphase cells; furthermore, the neoplastic and non-neoplastic cells do not differ significantly with respect to incidence of microvilli on their exposed surfaces. The incidence of ruffles and blebs and the heterogeneity of cell size and shape vary considerably yet do not correlate with neoplastic state. On the other hand, neoplastic cells characteristically show little flattening or spreading on the coverslip as compared with non-neoplastic. Also abundant lateral processes appear consistently in two of three neoplastic lines as compared with only two of six non-neoplastic lines.

Study 2. Microfluorimetry to study the metabolism of intact cells (Collaboration with Dr. Kohen). NADH and NADPH but not NAD^+ and $NADP^+$ exhibit fluorescence when excited by uv light of wavelength around 340mu. A few molecules of glucose-6-PO_4 are gently introduced into a single cell by microelectrophoresis. Changes in fluorescence can then be followed and the compartments of metabolism in the cell delineated. Neoplastic and non-neoplastic cells of common single cell origin are being compared. Also fluorescent carcinogens will be introduced to follow their metabolism.

Proposed Course:

The films prepared for Study 1 are being analyzed to quantitate growth rates, migration rates, and rates of cell shape (conformational) change as possible parameters characterizing neoplastic cells as compared with their non-neoplastic counterparts.

Two cytologic criteria associated with neoplastic change (See Serial No. 5228) are increased basophilia and reduced flattening or spreading of cells which we associate with loss of surface-substrate dependence (capacity for growth in suspension). The basophilia presumably results from increased numbers of ribosomes. Quantitative studies on ribosomal and protein content of the paired lines during log and stationary growth will be made by sucrose density analyses. The mechanism of cell shape change and its relation to microbubule formation is being studied (See Serial #3264).

Publications:

Sanford, K.K., Handleman, S.L., Fox, C.H. et al.: Effects of Chemical Carcinogens on Neoplastic Transformation and Morphology of Cells in Culture; J. Nat. Cancer Inst. 53 1647-1659, 1974.

Sanford, K.K.: Biologic Manifestations of Onocogenesis in Vitro: A Critique. J. Nat. Cancer Inst. 53: 1481-1485, 1974.

Keyword Descriptors: The conversion of normal to cancer cells in tissue culture.

Z01 CBD 3234A-03 LB
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Primary biochemical alterations leading to neoplastic conversion.

Previous Serial No.: Same

Principal Investigator: R. R. Gantt

Other Investigators: K. K. Sanford, Marilyn Taylor, Paolo Izzo

Man Years:
Total 4½
Professional 2½
Other 2

Project Description: To elucidate the primary chemical alterations which are necessary biochemical changes leading to neoplastic transformation and to devise methods to detect and control the transformation based on these chemical alterations.

Objectives: To identify primary changes in the methylation of cellular and viral nucleic acids during neoplastic transformation and to exploit these changes to develop an assay for, and possible control of, neoplastic transformation in vitro.

Methods Employed: In addition to the usual laboratory techniques and innovations, we use cloned, paired cell lines to control the wide variations among cell lines and even in the same cell line at different culture generations. These paired lines are described by Dr. Katherine Sanford, Project #______.

Major Findings:

1. The N^2- guanine RNA methyltransferase associated with avian myeloblastosis virus has been partially purified and characterized. The specific site of phenylalanine transfer RNA which is methylated has been determined. This information may enable us to find out if the enzyme is unique to virus infected cells and whether it is important to the virus life cycle.

2. Specific RNA methylases are being purified from chick embryo cells to acquire highly purified antibodies which will be used to develop sensitive assays for the specific proteins in single cells and tissues. To date, one methylase has been purified by ion exchange and affinity chromatography. This approach is being developed to monitor specific methylases which may be implicated in cell differentation and malignant conversion.

3. Evidence from Cot curves of chick DNA indicates that the distribution of 5-methylcytosine (a minor base in eukaryotes) is significantly higher in the repetitive sequences of the DNA. This information may help provide insight as to the biological function of eukaryotic DNA methylation.

Significance: The importance of nucleic acid methylation has recently been emphasized by the discovery of post transcriptional mRNA modifications which are required for translation of the message. Work on the biological functions of the numerous specific methylases present in cells has been hindered by lack of specific, sensitive techniques for studying the events. This program is designed to develop the requisite techniques and apply them to the problems of regulation and malignant conversion of cells where nucleic acid methylation has been so often implicated.

Proposed Course of Investigation:

1. Establish whether the N^2-guanine methylase is essential for the virus life cycle.

2. Identify differences in the paired cell lines and establish whether they are general.

3. Compare mitochondrial methylases of the paired cell lines. Preliminary evidence in our laboratory has shown that mouse liver and Leydig tumor cell mitochondria are quite different with respect to methylases and for decades mitochondrial lesions have been implicated in malignant conversion.

4. Continue to purify selected methylases to prepare antibodies for single cell fluorescent assays and quantitation of specific enzymes on small numbers of cells.

Publication:

1. Gantt, R.R.: Are nucleic acid modifications involved in neoplastic transformation? *J. Natl. Cancer Inst*, 1505-1508, 1974

2. Gantt, R.R. and Smith, G.H.: RNA methylase activity in virus-infected normal and neoplastic mouse mammary tissue. In "The Biochemistry of S-Adenosylmethionine", edited by E. Borek, F. Salvatore, F. Schlenk, G. Williams-Ashman, and V. Zappia. Columbia University Press, New York, 1975, In Press.

3. Gantt, R., Smith, G.H. and Julian, B.: Virion-associated and cellular RNA methylase activity in normal and neoplastic mammary tissue from MMTV infected and uninfected mice. In Press, *Cancer Res.*

4. Longfellow, D.C., Gantt, R. and Smith, G.H.: DNA methylation by particulate fractions from mammalian nuclei in S phase. Submitted to *Experimental Cell Research*.

Keyword Descriptors: methylation/ nucleic acids/ malignant conversion/ methylase/ tumor virus.

Z01 CBD 3257-01 LB
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Factors influencing the growth and neoplastic conversion of mammalian cells in vitro

Previous Serial No.: Same

Principal Investigator: W. G. Taylor

Other Investigators: K. K. Sanford

Cooperating Units: R. Parshad, Department of Pathology, Howard University School of Medicine, Washington, D. C.

Man Years:
Total: 3
Professional: 5/6
Other: 2 1/6

Project Description:

1. Effect of inoculum size and saturation density on development of malignancy in rapidly subcultured cloned cell lines.

2. Effect of lowered oxygen tension on plating efficiency of cultured mammalian cells.

3. Further analyses of a growth factor for mammalian cells in serum-free medium.

Objective: Manipulation of inoculum size to minimize cell-cell contact was reported as a means of preventing the acquisition of tumorigenic potential by nonneoplastic tissue culture cells. BALB/c embryo cells grown under conditions of extensive cell-cell contact (BALB/c-3T12) produced tumors in syngeneic hosts, whereas cultures initiated from the same primary cell pool but subcultured under conditions which minimize cell-cell contact did not give rise to tumors (Aaronson and Todardo, Science, 162:1024, 1969). Earlier, extensive observations in this laboratory were at variance with this report. Thus, studies were designed to test: (1) if spontaneous neoplastic transformation (NT) is enhanced by extensive cell-cell contact in vitro; (2) if, conversely, minimal cell-cell contact delays or prevents NT; and (3) if changes in growth rate and saturation density, morphologic and cytologic features, susceptibility to activated macrophages, and colony development in semi-solid suspension culture would correlate with the onset of NT.

Methods Used: Cell strains NCTC 7914 and 7915 were derived from single cell clones of a low passage C3H/f mouse embryo cell culture. Parent cultures at the 12th-13th passage level were each divided into two experimental sets; one set of cultures was routinely subcultured at 10^5 cells/T15 and became confluent in about 4 days, while the other set was subcultured at $4x10^5$ cells/T15 and became confluent at about 48 hours. Each set of cultures was carried on an alternating 3 and 4 day transfer schedule. Neoplastic transformation of the cultures was assessed by intraocular injection (Evans, et al., J. Natl. Cancer Inst. 32:89, 1962) During the course of the experiment, each strain was preserved by freezing. Subsequently, comparative analyses of growth rate, plating efficiency and saturation density, morphologic and cytologic alterations, susceptibility to activated macrophages, growth in agarose suspension culture, and glycolytic characteristics were performed on thaws of the frozen stocks.

Major Findings: (1) All cell strains, irrespective of the culture conditions used, became tumorigenic for syngeneic animals. However, cell populations carried at the lower inoculum level either required a longer period in culture before undergoing NT(as compared with the same clone carried at the higher inoculum size), or exhibited a longer tumor latent period. (2) Tumorigenic capacity, as assayed in vivo, correlated well with susceptibility to activated macrophages (see also NCI-3264), and cytologic alterations pathognomonic of in vitro NT(see also NCI-5228). An increase in saturation density or growth in semi-solid suspension culture was more closely related to time in culture and/or a short tumor latent period than with the onset of tumorigenic potential.

Significance and Proposed Course: (1) Culture manipulations which minimize cell-cell contact are not means sufficient to prevent spontaneous NT. A higher frequency of tumor development in vivo and a generally shorter tumor latent period were observed for cell populations carried under conditions of maximal cell-cell contact; however, an enhancement of NT, whether selective or inductive, would be difficult to prove from the data. (2) The data challenge: a) the reproducibility of the hypothesis, widely accepted, relating saturation density and the acquisition of neoplastic potential; b) the validity of the use of 3T3 cells as "normal" (i.e., nonneoplastic, nontransformed) cultures in cancer-related studies. Porter, et al (J. Cell Biol. 59:633, 1973) showed by SEM that 3T3 cells resemble endothelial cells in form and structure; subsequent to the initiation of this study Boone (Science, 188:68, 1975) developed a new in vivo assay method in which "normal" 3T3 cells gave rise to bloody hemangioendotheliomas; and c) the usefulness of growth rate and saturation density changes, or growth in semi-solid suspension systems as markers of NT.

One strain developed during this study showed a delay in the onset of NT, but also exhibited an array of cytologic and growth alterations. Studies are being designed to quantitate these changes and changes in serum requirements during the progression from the preneoplastic state to NT.

Project No. 2

Objective: Earlier studies showed enhanced colony development (growth) if the oxygen concentration of the culture milieu was decreased to 2% v/v (Taylor, et al, Expt. cell res 86:152, 1974). Studies were designed to: (1) test if enhanced growth with a gas phase containing 1-2% O_2 v/v was unique to fetal or embryonic cells, since most cells exhibiting this reponse were derived from embryonic or fetal tissue: (2) test if optimum plating efficiencies (PE's) varied as a function of the medium P_{O_2} and cell number planted; and (3) determine if vitamin E would enhance PE of human diploid WI-38 cells.

Methods Used: Test cells included: low passage adult human fibroblasts WilMac and BiRil(ATCC collection), Phase II WI-38, and mouse NCTC 8609 cells. Plating studies were done in pyrex T-30 flasks with Dulbecco-Vogt's modified MEM containing 10% v/v fetal bovine serum. Cultures were gassed with 10% CO_2 gas mixtures containing 1%, 6%, 12%, and 18% v/v O_2, balance N_2, or 5% CO_2:N_2. Vitamin E was emulsified in growth medium by blending.

Major Findings: (1) Low passage adult human fibroblasts developed 7-9 times more colonies with 1% O_2 in the gas phase than with the standard 10% CO_2:air. (2) In studies to date assessing colony development and graded medium P_{O_2} an inverse relationship was observed between dissolved oxygen in the plating medium and PE. Excellent PE's were seen, with as few as 100 cells and 0-1% O_2 gas phase (medium P_{O_2} = 40-50 mm Hg). (3) Vitamine E, and antioxidant reputed to extend the "finite life span" of human diploid fibroblasts (Packer and Smith, PNAS 71:4763, 1974), did not reverse the diminished colony development usually seen in plating studies with atmospheric (18% v/v) O_2. PE's observed with control and test cultures gassed with 1% O_2 were similar, indicating that Vitamine E is neither growth promoting nor inhibiting under the conditions used.

Significance and Proposed Course: In current plating methods cells are exposed to 18-20% O_2 v/v, either deliberately introduced as 10% CO_2:air or present as the residual gas. Other studies in this laboratory demonstrated that atmospheric O_2 enhanced karyotypic instability and, presumably, genic alteration. Thus, essential respiratory gases present in abnormal concentrations may be a factor in the development of heteroploidy and, perhaps, neoplastic transformation.

Additional studies are needed to: (1) confirm and extend the Vitamin E studies; and (2) explore the role, if any, of catalase and peroxidase in the survival and colony development of nonneoplastic cells. In the long term, additional research is needed in the development of specialized plating media for specific human cell types.

Project No. 3:

Objective: A broad range of problems are associated with the use of serum in tissue culture medium (Taylor, J. Nat. Cancer Inst. 53:1449, 1974).

Autoclavable, low molecular weight fractions (PD) of certain commercially available peptones promote the growth of several mouse, rabbit, monkey and some human cell lines. Studies were designed to: (1) complete earlier experiments regarding chemical analyses for specific in nutrients in PD, coupled with assessment of the growth requirements of peptone dependent cells by addition of selected nutrients to chemically defined medium (CDM); and (2) orientiation studies designed to purify and/or identify the growth promoting factor(s).

Methods Used: (1) Based on analyses of PD or literature reports on low molecular weight growth factors, a variety of nutrients were tested for growth promoting activity. Replicate cultures of RH-PD cells were used. (2) aqueos PD was passed through a 1x10 cm Chelex-100 column (Na^+ form), or chromatographed on a 40x1.5 CM-52 column with a stepwise 0.005-0.5M NH_4HCO_3 gradient, pH 8.3. Effluent fractions were lyophylized, reconstituted and sterilized, and tested for growth promoting activity as described above.

Major Findings: (1) Several growth-promoting nutrients failed to supplant the peptone requirement of RH-PD cells. (2) In studies to date, Chelex-100 treated PD (recovery = 70%), on a weight basis, promotes a cell population about ∿40% greater in number than control medium supplemented with untreated PD. In preliminary CM-52 chromatograms, moderate separation of UV-absorbing peaks was achieved but the material recovered (∿30% of starting material) was marginally active.

Significance and Proposed Course: The use of serum as a supplement to CDM is an expedient but biologically unsatisfactory practice. Serum is a source of chemical and experimental variability in vitro and can be the source of adventitions microbial contamination. Since serum is not an obligate requirement of mammalian cells in tissue culture, the development of better chemically defined media and/or biologically adequate serum substitutes is fundamental to progress in research and clinical endeavors (e.g. safer vaccine production) which use cell culture. Since PD is an autoclavable growth promoting serum-substitute for several long-term cell strains, certain immediate objectives are being pursued: (1) inactivation studies designed to provide positive, direct evidence that the active component(s) is a peptide (2) fractionation studies to further purify the growth promoting factor(s), (3) the use of these data to improve existing CDM, with emphasis on primary cell culture coupled with studies designed to assess the metabolic role of PD in the growth of peptone-dependent cells.

Publications:

1. Taylor, W.G., Richter, A., Evans, V.J. and Sanford, K. K. Influence of Oxygen and pH on Plating Efficiency and Colony Development of WI-38 and Vero cells. Exptl. Cell Res.86: 152-156, 1974.

2. Sanford, K. K., Gantt, R.R. and Taylor, W.G. Preface (New Horizons for Tissue Culture in Cancer Research) J. Natl. Cancer Inst. 53:1429, 1974.

3. Taylor, W. G. "Feeding the Baby" -- Serum and Other Supplement to Chemically Defined Medium. J. Natl. Cancer Inst. 53:1449-1457, 1974.

Keyword Descriptors: transform cells/ population density/ clonal growth/ oxygen/ serum-substitute/ tissue culture.

Z01 CBD 3263-01 LB
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Developmental research on new methods for the cultivation of very large numbers of human and other mammalian cells in vitro

Previous Serial No.: Same

Principal Investigator: W. G. Taylor, R. F. Camalier

Other Investigators: K. K. Sanford

Man Years:
Total: ½
Professional: 1/6
Other: 1/3

Project Description: Non-neoplastic and some neoplastic cells require a surface upon which they can attach, migrate, and divide. This study evaluated a new means of growing gram amounts of "substrate-dependent" mammalian cells in a single culture vessel.

Objectives: Efforts were made to: (1) develop a rapid, physiologic means for dispersing adherent cell populations from the growth surface, and (2) determine if a complete change of medium after 24 hours incubation, a high glucose concentration, or the use of insulin would promote more rapid growth of WI-38 human diploid fibroblasts.

Methods Used: The Dyna-Cell^R is a cylindrical vessel which houses a spiraled plastic growth surface. Cells and medium (1.6 - 1.7 l) are introduced through ports on top of the vessel, and the culture medium aerated and gently circulated by intermittent gassing with 5% CO_2 in air. Since the inner layers of plastic cannot be visualized, cell growth and the need for medium renewal are assessed indirectly by measurements of available glucose.

Major Findings:
1. Gram quantities of nonneoplastic, human diploid fibroblasts (WI-38) can be grown and recovered from a Dyna-Cell^R vessel provided: (a) an inoculum of 5-8x10^7 cells (∿0.5cc, packed cell volume) is used; (b) medium glucose is monitored to assess the need for a complete change of culture medium; and (c) unchilled cell populations are harvested with 0.125% v/v pancreatin-0.01% v/v versene. Cell populations recovered were >95% viable by trypan blue exclusion procedures.

2. With existing medium and methods a 20-5 fold increase in WI-38 cell number is achieved in 7-10 days. A protracted lag in glucose utilization, and presumably proliferation, is observed during the first 72 hours incubation. Neither a complete change of medium at 24 hours (to remove cell debris, residual pancreatin, etc.), the use of an enriched culture medium, nor the use of one IU of insulin per ml medium shortened the growth lag or significantly enhanced cell-yield.

Significance and Proposed Course: A single Dyna-Cell^R vessel can produce gram quantities of substrate-dependent cells for tumorigenesis assays, biochemical studies of cell products, membrains, organelles, etc., and will be used to produce large quantities of nonneoplastic cells which can be frozen as a reference stock for future experiments.

The proposed course includes: (1) design and testing of a modified, reusable vessel; (2) further assessment of the need for new medium formulations and of ways to eliminate costly medium renewals; and (3) use of the vessel to explore the effects of nonphysiologic variations in the culture milieu on neoplastic transformation.

Publications:

Manuscript submitted for publication.

Keyword Descriptors: mass culture/ tissue culture/ WI-38-substrate of dependent cells.

Z01 CBD 3264-02 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Spontaneous Neoplastic Change During Growth in Culture

Previous Serial No.: Same

Principal Investigator: R. W. Tucker

Other Investigators: K. K. Sanford, G. M. Jones and R. Gantt

Cooperating Units: M. S. Meltzer, Biology Branch, NCI,
A. C. Breuer, Behavioral Biology Branch, NICHD
H. Sato, Biophysical Cytology, Biology Dept.
University of Penna., Philadelphia, Penna.

Man Years:
Total: 1½
Professional: 1
Other: ½

Project Description:

Objectives: To study the behavorial changes which cells undergo during neoplastic change in culture in vitro.

1. To determine if neoplastic and non-neoplastic cells from the same single cell have different growth dependence on serum.
2. To define the relative susceptibility of neoplastic and non-neoplastic cells to BCG activated magophages.
3. To study the organization of microtubules and microfilaments in neoplastic and non-neoplastic cell.
4. To assess the ability of growth in agarose to differentiate between neoplastic and non-neoplastic cells.

Methods Employed:
1. The growth of neoplastic and non-neoplastic cells was measured in medium supplemented with different amounts of serum. Accompanying changes in intracellular concentrations of C-AMP were measured by radioimmunoassay.
2. The cytotoxic reactions of BCG-activated mouse peritoneal macrophages was tested on neoplastic and non-neoplastic cells.
3. The organization of microtubules and microfilaments was measured by the birefringence of these structures in living cells as visualized in the polarization microscope.

4. The growth of cells as discrete colonies in agarose was measured in the light microscope.

Major Findings:

1. Neoplastic and non-neoplastic cell descendents from the same cloned single cell have the same growth curves in the same medium. Preliminary findings also suggest that the cell lines derived from the same single cell have the same intracellular content of cyclic AMP when growing at the same rate.
2. a) Neoplastic cell lines, regardless of species are more susceptible then their non-neoplastic cell line counterparts to the cytotoxity of BCG activated mouse peritomeal cells (Tucker, R. W., Meltzer, M. S. and Sanford, K. K., the differential susceptibility between tumorigenic and nontumorigenic paired cell lines to BCG-activated mouse peritoneal macrophages; abstract FASEB 1974).

   b) The release of tritiated thymidine from the target cells successfully quantitates the cytotoxic response of neoplastic cells caused by BCG activated macrophages (Meltzer, M. S., Tucker, R. W., Sanford, K. K. and Leonard, E. J., Interaction of BCG activated macrophages with neoplastic and non-neoplastic cell lines in vitro: Quantitation of the cytotoxic reaction by release of 3H-thymidine from prelabeled target cells. In Press, JNCI).

   c) Films of the interaction of BCG activated macrophages with neoplastic and non-neoplastic cells reveals the dynamics of the cellular interaction (Meltzer, M. S., Tucker, R. W., Breuer, A. C., Interaction of BCG activated macrophage cell lines in vitro: Cinemicrographic Analysis, in press, J. Immonology).

   d) Preliminary results suggest that the neoplastic cells susceptibility to BCG-activated macrophages may occur 50 to 100 days after the increase in tumorgenicity occurs.
3. Birefringence is used to measure the amounts of oriented fibillar material in living cells.

   A. Effects of dibutyrl cyclic AMP on Mitotic and Meiotic Spindle Microtubules:
      1. Preliminary results indicated that 10^{-3}M dibutyrl cyclic AMP causes an early increase in the birefringence of the mitotic spindle of the fertilized sea urchin egg.
      2. Dibutyrl cyclic AMP causes no increase, and probably causes a decrease in the birefringence of the metaphase-arrested meiotic spindle of the unfertilized star fish egg.
      3. 10^{-3}M dibutyrl cyclic AMP causes no change in the birefringence of the mitotic spindle in CHO.

   B. Effects of dibutyrl cyclic AMP on cytoplasmic birefringence in the CHO cell.
      1. 10^{-3}M dibutyrl cyclic AMP increased the cytoplasmic birefringence along the CHO cell periphery within 1-2 hours. These birefringence changes were associated with elongation of the CHO cell into a bipolar morphology. These same birefringence and shape changes occurred in the cell at high cell density.

2. Neoplastic cells grow much better in agarose than do non-neoplastic cells. Non-neoplastic cells produced no colonies, while the neoplastic cell line produced many colonies in the agarose. However, the size and number of the colonies did not correlate with the tumorgenicity of the neoplastic cell lines.

Significance and Proposed Course:

1. Changes in serum dependence for growth in vitro do not necessarily accompany neoplastic changes in vitro. Moreover, the cyclic AMP content is the same in both neoplastic and non-neoplastic cell lines growing at identical rates. If cyclic AMP content does play a role in the expression of a neoplastic state of a cell population in vitro, then it must determine differences in cell population behavior only in the stationary, plateau phase of growth in vitro, and not in the log phase. Accordingly, these preliminary results will be confirmed and then extended to a more detailed analyses of the plateau phase of growth.

2. BCG-activated macrophages kill neoplastic cell lines much more readily than they affect non-neoplastic cell lines, regardless of tissue or species type. In using this macrophage-target cell interaction as an in vitro assay for neoplastic cells, one must know if the reaction correlates with the development of tumorgenicity in vitro. Our preliminary results indicate that the macrophage killing does not correlate in time with this development of tumorgenicity in vitro. If confirmed these results will restrict the use of BCG activated macrophages as an in vitro assay. A malignant macrophage cell line, P388, will be tested in this assay to determine if a long term cell line of macrophages can be activated in vitro or in vivo to selectively kill neoplastic cells. The possibility that the inhibition of DNA synthesis of neoplastic cells by BCG-activated macrophages also correlates with tumorgenicity will be tested. Preliminary evidence inciates that DNA synthesis of neoplastic cells is inhibited by BCG activated macrophages within 2-4 hours.

3. Dibutyrl cyclic AMP has been suggested to increase the assembly of cytoplastic microtubules in CHO cell. The increase polymerization of microtubule may be a characteristic of the non-neoplastic state in fibroblast cells. Preliminary work suggests that DBC affects cytoplastic microtubules much more readily than it affects mitotic spindle microtubules in CHO. Therefore, the control of assembly of microtubules in the cytoplasm must be different from that of the microtubules in the mitotic spindle. Furthermore, the control of the cytoplasmic fibrillar system may be more closely linked to neoplastic behavior than does that of the mitotic spindle. This work will be pursued in the following projects:

a) Verify that the cytoplasmic birefringence in CHO cells, with dibutyrl cyclic-AMP and high cell density, is microtubules and/or microfilaments by E. M. These observations would then be extended to neoplastic and non-neoplastic cells derived from a single cell.

b) Verify that dibutyrl cyclic AMP causes an increase in the early birefringence of the mitotic spindle in marine cells.

c) Determine if agents that affect the birefringence of mitotic spindle also affect the birefringence of cytoplasmic fibrillar systems.

4. The inability of non-neoplastic cells to grow in agarose or suspension culture may be an expression of the substrate dependence of non-neoplastic cells. Hydrophobic surfaces will be used in an attempt to extend this substrate dependence phenonema to a medium in which it can be more readily analyzed.

Keyword Descriptors: spontaneous neoplastic change/ serum dependence
BCG macrophages/ agarose/ microtubules.

Z01 CBD 5228-01 LB
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Cytology of Neoplastic Transformation In Vitro

Principal Investigators: S. Handleman, K. K. Sanford

Other Investigators: R. W. Tucker, G. M. Jones, F. M. Price, C. H. Fox, C. U. Garrison, R. F. Camalier

Cooperating Units: None

Man Years:
Total: 2 1/8
Professional; 3/8
Other: 1 3/4

Project Description:

Objective: To identify any cytologic characteristics of cells in culture that may be directly or indirectly associated with their neoplastic transformation in vitro and then to utilize those cytologic characteristics for studying the induction of neoplastic transformation by chemical carcinogens, as well as for following the progressive, often very subtle change associated with the spontaneous neoplastic transformation of cells in vitro.

Methods Employed: A combination of cytologic criteria previously described and validated for C_3H mouse cell cultures is used to study individual cells at high power as well as colony morphology at low power on a 24-48 hour cover-slip preparation. Colony morphology is also used to analyze cell populations on 10-13 day petri dish or T25 flask preparations.

Cytologic Studies on "Spontaneously" Transformed Cells:
Study 1: The cytology of cells undergoing neoplastic transformation in culture.

The objective is to study the progressive cytologic changes of embryo cells of human, rat, hamster and mouse origin during long-term culture and to establish more quantitative, objective cytologic criteria for neoplastic transformation of cells in vitro and a systematic approach to in vitro diagnosis of neoplastic state. This study was outlined in last year's annual report.

In an attempt to make some definite conclusions about the applicability of certain in vivo cytologic criteria to cells in culture and to explore the feasibility of quantitation certain morphologic properties of colonies in vitro, 24-48 hour cytologic preparation are being further analyzed. The colonies on a 24 hour cytologic preparation are the product of spreading and migration of cells from a clump of cells derived from a monolayer culture. It appears that there is a correlation between the progressive loss of parallel orientation of cells migrating out of a clump and increasing tumorigenic potential. Coverslip preparations are being re-examined in order to classify each individual colony based on the degree of parallel orientation exhibited by the cells. By photographing each colony, placing a transparent overlay on the colony and marking the long axis of each cell, it is possible with a tool consisting of a movable arm attached by a pin to the center of the colony and bearing a sliding protractor and ruler to measure the angle of each cell relative to the radial axis of the colony. By this means histograms of the angles in the various proximal and distal zones of the colony can be displayed.

Further analysis of the cytologic preparations and statistical interpretation of the data are required before the feasability of this approach for computer programming and its usefulness for diagnosis can be more fully determined.

Study 2: The use of colony morphology for the diagnosis of neoplastic cells in culture.

The objective of this study is to use colony morphology to analyze cell populations and to give some estimate of what percentage of neoplastic cells are found in a cell population. This study was outlined in last year's annual report. It has been completed and a manuscript is in progress.

Study 3: The cytology of rat liver epithelial cells.

The objective of this study is to identify any cytologic characteristics of cultured rat liver epethelial cells that may be directly or indirectly associated with their neoplastic state. Because the cytologic criteria of malignancy as applied to fibroblast-like cells in culture may not be wholely applicable to cultured epithelial cells, several sets of nonneoplastic and carcinogen neoplastically transformed rat liver epithelial cells are being prepared for cytologic study.

Careful evaluation of the cytologic preparations will be needed before any definite conclusions about the applicability of certain cytologic criteria to epithelial cells can be made.

Other Activities of the Past Year Were:

1. The development of a line of rat liver epithelial cells.

The objectives of this study were: To develop a method for setting up cultures of epithelial cells from adult rat livers and determine the culture conditions needed for their optimum growth; to collect and save the supernatent medium from the cultures and see how its composition changes during long-term culture; to apply chemical carcinogens to the cultures and follow their metabolism by the cells. This study is still in progress.

2. The development of a rapid routine chemical assay to test cell cultures for the presence of PPLO.

The objective of this study is to develop a rapid routine chemical assay to test cell cultures for the presence of PPLO using an enzymatic assay for uridine phosphorolase reported to be specific for microorganisms. To date no false negatives tests have been seen although several false positive tests (as compared to cultured PPLO tests) have been seen. However, this enzymatic assay has potential future promise as a first screen for PPLO. The difficulty is adequate evaluation of false positive tests.

Keyword Descriptors: Cytologic characteristics of cells undergoing neoplastic transformation during long-term culture.

Z01 CBD 5239-01 LB
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinomatous change in human cells studied *in vitro*.

Previous Serial No.: None

Principal Investigator: Cecil H. Fox

Other Investigators: K. K. Sanford, F. M. Price, G. M. Jones, C. U. Garrison

Cooperating Units: K. K. Sanford (Res. Biologist), F. M. Price (Res. Biologist), G. M. Jones (Microbiologist), C. M. Garrison (Laboratory Technician), all of the Cell Physiology and Oncogenesis Section, Laboratory of Biochemistry: J. A. Dvorak, Lab. of Parasitic Diseases, NIAID; J. K. Selkirk, Chemistry Branch, NCI; D. H. Reese, Lung Cancer Branch, NCI; M. H. Cottler-Fox, Viral Oncology Branch, NCI; B. K. Wetzel and W. R. Levis, both of Dermatology Branch, NCI; R. Parshad, Howard University Medical School; T. Caspersson, Karolinska Institutionen, Stockholm Sweden; L. Koss, Department of Pathology, Albert Einstein School of Medicine, New York; J. Jacobs, U. of Massachusetts Medical School, Worcester, Mass.

Project Description:

Objective: To study the changes that occur in differentiated human epithelial cells accompanying transformation using morphological, chemical and physiological parameters. Further, to establish a system for transformation of human cells at will and to use such a system for estimating the liklihood of neoplastic transformation *in vivo*.

Methods Employed: The methods to be employed will be described under each study heading.

Study 1. Neoplastic transformation of human epidermal cells. This study is the central portion of the project. Epidermal cells are being isolated into culture by Mr. F. M. Price using techniques he has developed over the past year. We are now able to obtain growing epidermal cell cultures from human skin or gingiva with remarkable regularity. These cultures are maintained as primary cultures which are entirely adequate for the types of studies we are now conducting. The skin cells are treated with a variety of carcinogens. At present we are using polycyclic hydrocarbons with our interest centered on benzo(a)pyrene, a carcinogen known to be widespread in the environment. Recent experiments in cooperation with Dr. J. K. Selkirk have demonstrated the metabolism of benzo(a)pyrene to epoxides, arylhydroxides, and quinones, an event not previously described in cultured human epidermal cells. Since polycyclic hydrocarbon metabolism is a necessary step in carcinogenesis in epithelial cells, we feel this to be an encouraging step forward. We are continuing studies of carcinogen metabolism with special emphasis on the unusual pattern of metabolites that are produced by epithelial cells. Fibroblasts, the easiest human cells to culture, do not metabolize the polycyclic hydrocarbons as far as is now known. We are in addition using mixtures of polycyclic hydrocarbons to study the induction of arylhydrocarbon metabolizing enzymes in human prostate cells isolated for us by Dr. R. Parshad from clinical material available to him.

A persistent problem has been in obtaining healthy human skin. Most of our supply has been furnished by local surgeons from patients undergoing plastic and reconstructive surgery. We are fortunate in having the cooperation of Dr. W. R. Levis who is now providing us split thickness skin sections in a collaborative study.

It should be noted that human skin is one of the most difficult systems to study, both because it is extremely fastidious in its growth requirements, and because it is far more difficult to transform than other mammalian cells. This research is time consuming, tedious, and will be of long duration, but in the light of 85% of human cancer being due to carcinoma we believe that the difficulties are bearable and understandable.

Study 2. Effect of physical carcinogens.

In studying the effects of asbestos and glass fibers on cultures of rodent mesothelial cells in culture we have been fortunate in collaborating with G. M. Jones who has found that cells exposed to particulate materials such as asbestos behave in a peculiar fashion in that the particles are adherant to the surface of the treated cells, that the particles stick to a particular point on the cell surfaces, and that when cells divide with particles adhering to them, the particles go with only one of the cells, the other being naked. We intend to pursue this matter at greater length using other cell types.

In addition, after noting that in humans who are constantly exposed to particulate carcinogens, the incidence of broncogenic carcinoma is dramatically increased when the individuals smoke cigarettes we will be exposing human skin and possibly rodent mesothelial cells to carcinogen coated asbestos particles.

Study 3. Transformation of Transitional Epithelium.

In collaboration with D. H. Reese, we are studying the morphological changes associated with neoplastic transformation in rat bladders following treatment with chemical carcinogens. We are seeking to determine several points of interest including a light microscopic study of the morphological events occuring in cultured transitional epithelium in the carcinogenic sequence. Cultures of epithelium will be isolated as primaries and an attempt will be made to discover morphological characters of transformation. With transitional epithelium we are also interested in studying three essential features of bladder carcinoma which we hope to apply to human cells when suitable clinical material becomes available. These are: a) whether or not asymmetric membranes, virtually diagnostic for transitional epithelium, are synthesized in culture and whether or not these membranes are found in transformed epithelial cells; b) whether transitional epithelium continues to form desmosomes in culture and if so, the rate and conditions for formation of these interesting structures; c) whether transformed transitional epithelium continues to form "micro-ridges" at the cell surface following transformation.

These studies are being conducted with the additional cooperation of two electron microscopists, M. H. Cottler-Fox and J. Jacobs.

Study 4. Regulation of Lactic Acid Metabolism in Cultured, Neoplastic and Embryo Cells

A fundamental characteristic of tumor cells and cells in culture has been that glucose is metabolized to two moles of lactic acid with very little pyruvate being formed, and with lactate accumulating as a non-metabolizable pool. We are at present studying this phenomenon and have made considerable progress in methodological technology. First, we are interested in the relationship between oxygen supply available to the cells and their tendency to ferment rather than respire. Cells have been cultivated at varying oxygen concentrations with varying population densities and on different media. The culture fluids have been systematically collected and are now being analyzed for the amounts of lactate and glucose they contain. We have developed an interesting analytical method for doing this. A 10 ul sample of culture medium is used for the simultaneous determination of glucose, lactate, pyruvate, all of the physiological alpha keto acids, inositol and galactose. The sensitivity of the method is between one and ten picomoles of metabolites. About thrity samples can be run per day. Development of these methods will be greatly implemented by the recent renovation of a lab bench allowing permanent installation of the gas chromatograph used in these studies.

In addition to studying lactate production in established cell systems, we are also engaged in attempting to determine the physiological sequence of lactate production in newly established human cell cultures in order that we may start a more detailed biochemical examination involving isolation of enzymes of pyruvate metabolism.

In addition to studying lactate metabolism in normal cells, we are also making comparisons between neoplastic and non-neoplastic populations. It now appears that attempts to compare these populations require extensive collateral information such as cell cycle data and population density values. Another major point that is now being investigated is the amount of regulation of the lactate pathway by energy charge relationships in cell systems. This will be investigated as soon as suitable methods for determining ATP-ADP-AMP ratios are developed.

Study 5. Morphology of Transformation in Human and Rodent Cells.

In collaboration with K. K. Sanford and J. A. Dvorak we have found that there are certain morphological characteristics of transformed rodent fibroblasts that can be quantitatively described using light microscopy. These characteristics include cell thickness, projected area, and relationships between cytoplasmic RNA and protein concentrations. In order to pursue and amplify these observations we have obtained the collaboration of Prof. T. Caspersson who has consented to allow us to use his integrating interferometer and automated microscopes, and with Dr. L. Koss who is providing us with pathologic interpretation of cells in conventional Papanicoulau preparations.

Miscellaneous Studies:

In addition to the above, we are in the process of completion of several projects begun last year including: A short-term study on average DNA chain length from cells grown in horse serum compared to cells grown in fetal calf serum, this being done in collaboration with Dr. K. W. Kohn. We are concluding a study of the use of ruthenium red as a means of detecting neoplastic transformation in cultured cells, and we are completing a study begun some time ago with B. K. Wetzel on the formation of microvilli by cultured cells.

Keyword Descriptors: human carcinogenesis/ chemical carcinogenesis in vitro/ gas liquid chromatography/ glycolysis/ quantitative microscopy/ transformation/ epithelium/ carcinomatosis in vitro/ polycyclic hydrocarbons/ physical carcinogenes/ transitional epithelium/ urothelium/ cytomorphology/ lactate metabolism.

Z01 CBD 5222-06 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individul Project Report
July 1, 1974 through June 30, 1975

Project Title: Mutations in the first structural gene of the histidine operon which alter regulation of the operon

Previous Serial No: None

Principal Investigator: Marilyn Meyers

Other Investigators: Robert Goldberger

Cooperating Units: Mark Levinthal, Department of Biological Sciences, Purdue University

Man Years:
Total: 0.4
Professional: 0.4
Other: 0

Project Description:

Objectives: On the basis of previous work done on the kinetics of repression of the histidine operon in Salmonella typhimurium, we suggested that the first enzyme for histidine biosynthesis plays a previously unrecognized role in control of the histidine operon. A prediction of this hypothesis is that certain mutations in the first structural gene (G gene) of the operon would lead to alterations in the structure of the first enzyme which affect regulation of the histidine operon. Although a number of mutations affecting the catalytic function of this enzyme are known, none of these affects the process of repression. We have been attempting to find mutations in the first structural gene which do alter the repression process.

Methods Employed: We developed a procedure which allowed the direct selection of feedback resistant mutations in the G gene of episomes carrying the histidine operon. Twenty feedback resistant episomes were isolated. Several were found to produce a G enzyme highly resistant to inhibition by histidine. Using these episomes to make merodiploid organisms, we were able to demonstrate that the effect of the G enzyme on repression of the histidine operon was trans dominant (see Project Report NIAMDD-LCB-14). The feedback resistant episomes is not under repression control. This is the first isolation of the consistitutive G gene mutations predicted by our hypothesis that the G enzyme plays a direct role in repression of the histidine operon.

This episome has been integrated into the chromosome of several strains carrying deletions of the histidine operon. The resulting strains exhibit the same patterns, i.e., the levels of the histidine enzymes are approximately 4X derepressed and are not under histidine control. The episome carrying the altered G gene has been transduced into his T and his W mutants of Salmonella and in all cases the enzyme levels are fixed at the 3X level. The same result was obtained when the episome was transduced into an E. coli strain which had the his operon deleted.

Major Findings: An episome bearing the histidine operon containing a mutation to feedback resistance has been isolated. The strain carrying this episome is not under repression control. This finding supports the hypothesis that the G enzyme plays a direct role in repression of the histidine operon.

Significance to Cancer Research: Alterations in the first enzyme for histidine biosynthesis which affect repression of the histidine operon constitute proof of our hypothesis that a biosynthetic enzyme may have regulatory properties which allow it to control its own synthesis as well as the synthesis of the other biosynthetic enzymes encoded in the same operon.

Proposed Course of Research: Terminate after publishing results.

Publications: None.

Keyword Descriptors: histidine biosynthesis/ gene regulation.

Z01 CBD 5223-06 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interaction between the first enzyme for histidine biosynthesis and His-tRNA

Previous Serial No.: None

Principal Investigator: Roger Deeley

Other Investigators: Tikva Vogel, Robert Goldberger, Marilyn Meyers and Kathleen Mullinix.

Cooperating Units: None

Man Years:
Total: 4
Professional: 2½
Other: 1½

Project Description:

Objectives: On the basis of our previous work on the kinetics of derepression of the histidine enzymes we postulated that the first enzyme for histidine biosynthesis plays a previously unrecognized role in repression of the histidine operon in *Salmonella typhimurium*. Because it has been known for some time that his-tRNA is required for repression of the histidine operon, it appeared to us that one test of our hypothesis is to determine whether the first enzyme for histidine biosynthesis interacts with his-tRNA. Using crude tRNA, we previously found that the enzyme had a high affinity for tRNA and that it bound his-tRNA in preference to tRNA which had been aminoacylated with any other amino acid. In the present study, we used various partially purified species of tRNA, to test the question of whether the enzyme binds the aminoacylated form of his-tRNA in preference to the deacylated form.

Methods Employed: Partially purified preparations of various species of tRNA were obtained from Dr. Donald Kelmers of the Oak Ridge National Laboratory. In addition, tRNA^{His} was purified to homogeneity from *Salmonella typhimurium* (both wild type and *T*-mutant) by the published procedure. The *G* enzyme and histidyl-tRNA synthetase were purified by the published procedures. Binding of the *G* enzyme to tRNA was studied by using the nitrocellulose filter binding technique of Yarus and Berg.

Major Findings: We found that for species of tRNA other than tRNA^{His}, the G enzyme does not distinguish between the aminoacylated and deacylated forms. In contrast, the G enzyme binds aminoacylated histidyl-tRNA in preference to deacylated tRNA^{his}. Thus, the enzyme not only binds the histidine species of tRNA in preference to all other species, but also binds the aminoacylated form of histidine tRNA preferentially. The possibility that histidyl-tRNA carries out it regulatory role as a complex with the G enzyme is consistent with these findings. Binding studies with his-tRNA from the T mutant demonstrated that the G enzyme does not differentiate between mutant and normal his-tRNA, in terms of its ability to bind either the acylated or deacylated forms of the tRNA when the enzyme is in either its active or feedback-inhibited states.

Significance to Cancer Research: The finding that his-tRNA, a macromolecule known to be required for repression of the histidine system, binds with a very high affinity to the first enzyme of the histidine biosynthetic pathway, may be an important clue to understanding the mechanisms which regulate biosynthetic metabolic pathways.

Proposed Course of Research: Terminated.

Publications:

1. Goldberger, R. F., Deeley, R. G. and Mullinix, K. P. M.: Regulation of gene expression in prokaryotic organisms. In Ernst W. Caspari (ed.): Advances in Genetics, Vol. X. New York, Academic Press, 1974. In Press.
2. Deeley, R. G., Goldberg, R. F., Kovach, J. S., Meyers, M. and Mullinix, K. P.: Interaction between phosphoribosyltransferase and purified histidine tRNA from wild type *Salmonella typhimurium* and a derepressed *hisT* mutant strain. *Nucleic Acids Research*, in press.

Keyword Descriptors: histidine bioynthesis/ his-tRNA/ gene regulation.

Z01 CBD 5224-06 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The effect of transfer RNA on the activity of G enzyme, the first enzyme in the histidine pathway

Previous Serial No.: None

Principal Investigator: Robert F. Goldberger

Other Investigators: Tikvah Vogel

Cooperating Units: Henry Kronenberg, NIAMDD-LCB

Man Years:
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: Histidine inhibits the G enzyme, the first enzyme in the histidine biosynthetic pathway in *Salmonella typhimurium*. Charged tRNA^{His} plays a role in repressing the histidine operon. G enzyme may play a role in gene regulation as well, and is known to bind specifically charged tRNA^{His}. We wanted to see if tRNA^{His} has any specific effect on the catalytic activity of G enzyme itself.

Methods Employed: A sensitive new assay for G enzyme was developed because the presence of nucleotides interfere with the standard spectrophotometric methods. The G enzyme catalyzes the reaction:

PRPP + ATP PRATP + PPi.

The assay uses the fact that ^{14}C-PRPP does not bind to charcoal at acid pH, but ^{14}C-PR-ATP does. Purified tRNA species were purified by reverse phase chromatography and were aminoacylated using standard techniques.

Major Findings: At very low concentration, tRNA partially inhibits G enzyme. There was no inhibition of G enzyme from a mutant with altered G enzyme feedback sensitivity to histidine. Preliminary studies show no specificity for charged or uncharged tRNA^{His}. ATP and PRPP reverse both the binding and the inhibition by tRNA.

Significance to Cancer Research: G enzyme--as the first enzyme in the histidine operon and pathway--may take part in histidine regulation on both the genetic and enzymatic level. Interaction with tRNA may shed light on the growing field of protein-nucleic acid interactions.

Proposed Course of Research: Terminated.

Publications: Henry M. Kronenberg, Tikvah Vogel and Robert F. Goldberger. A New and Highly Sensitive Assay for the ATP Phosphoribosyltransferase that Catalyses the First Step of Histidine Biosynthesis. Analytical Biochemistry, In Press.

Keyword Descriptors: phosphoribosyltransferase/ histidine biosynthesis/ G enzyme.

Z01 CBD 5225-06 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interaction between the first enzyme for histidine biosynthesis and Ø80d*his* DNA

Previous Serial No.: None

Principal Investigator: Marilyn Meyers

Other Investigators: Tikva Vogel, Roger Deeley and Robert F. Goldberger

Cooperating Units: None

Man Years:
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: We previously proposed that the first enzyme for histidine biosynthesis, the *G* enzyme, plays a role in regulation of the histidine operon. If this enzyme acts at the genetic level, it would be expected to interact with some regulatory element in the DNA of the histidine operon. The recent isolation of a phage carrying the histidine operon in place of some of its own genes has made it possible to test this possibility.

Methods Employed: Radioactively labeled and unlabeled DNA were isolated from Ø80d*his*. Interactions between these preparations of DNA and the *G* enzyme were studied by the filter binding assay used by Riggs and Bourgeois to study the binding of the *lac* repressor to the lactose operon. Competition experiments were done using cold Ø80DNA, cold Ø80d*his* DNA, and cold Ø80d*his* carrying a deletion mutation in the operator region of the *his* operon, to determine if these preparations of DNA also bind to the *G* enzyme.

Major Findings: The enzyme binds Ø80d*his* DNA. This binding is completely inhibited by cold Ø80d*his* DNA. However, cold Ø80DNA does not inhibit, and the cold Ø80d*his* operator deletion DNA does not inhibit. These results indicate that the *G* enzyme binds to the regulatory region of the *his* operon DNA.

Significance to Cancer Research The finding that an enzyme may serve as a regulatory protein, controlling the rate of its own biosynthesis, may be of general importance in understanding the mechanism of repression control.

Publications: Submitted, not yet accepted.

Keywords Descriptors: gene regulation/ histidine biosynthesis.

Z01 CBD 5238-06 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effect of Estrogen on Gene Expression in the Avian Liver.

Previous Serial No.: None

Principal Investigator: Robert F. Goldberger

Other Investigators: Kathleen P. Mullinix, Roger G. Deeley, Waldemar Wetekam and Marilyn Meyers.

Cooperating Units: None

Man Years:
Total: 4
Professional: 3
Other: 1

Project Description:

Objectives: To elucidate, with the aid of purified cell-free systems, the molecular mechanisms involved in the induction of egg yolk phosphorproteins in the avian liver, by estrogen.

Methods Employed: The egg yolk proteins, phosvitin and lipovitellin, have been purified and used as antigens. The antibodies produced in response to immunization with lipovitellin have been used to purify, by affinity chromatography, the egg yolk phosphoprotein precursor, vitellogenin. Structural studies on this precursor are in progress. A cell-free system, using a rabbit reticulocyte lysate, has been developed, which in conjunction with specific immunoprecipitation techniques allows the quantitation of messenger-RNA specifying vitellogenin. Indirect immunoprecipitation of polysomes synthesizing vitellogenin, followed by chromatography of the immunoprecipitated polysomal RNA on poly-(U)-Sepharose, has been used to partially purify the messenger-RNA specifying vitellogenin.

Major Findings: The egg yolk phosphoproteins are synthesized as a single, large precursor, vitellogenin, of M.W. 460-480,000. The complex consists of two subunits each of mol. wt. 220-240,000. It has been demonstrated by immunoprecipitation that up to 10% of the liver polysomes become involved in thesynthesis of vitellogenin following estrogen treatment of the rooster. Present results suggest that without estrogen treatment the rooster does not normally synthesize vitellogenin. Vitellogenin has been synthesized *in vitro* from RNA isolated from the liver of estrogen-treated roosters. Messenger RNA specifying vitellogenin has been purified more than 600-fold from crude polysomal RNA and translated in a cell-free protein synthesizing system.

Significance to Cancer Research: The system described offers the possibility of examining at a molecular level the involvement of steroid hormones in the regulation of gene expression and cytodifferentiation. The results of such a study on normal tissue may provide an insight into the causes of breakdown of such control mechanisms in malignant tissues.

Proposed Course of Research: The techniques that have been developed will be used to purify to homogeneity the messenger RNA specifying vitellogenin. This purified message will be used as a template from which to synthesize radioactive complementary DNA. "Probe" DNA prodced by this method will be used to detect vitellogenin messenger-RNA synthesized in vitro, using preparations of chromatin and RNA polymerase from the livers of estrogen treated and untreated roosters.

Keyword Descriptors: gene expression/ estrogen/ egg protein.

Z01 CBD 5240-01 LB
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The synthesis of vitellogenin by avian liver cells in culture

Previous Serial No.: None

Principal Investigator: Joseph H. Highland

Other Investigators: Junita Eldridge and Robert Goldberger

Cooperating Units: Dr. G. E. Grieninger, Rockefeller University, New York, N. Y., 10021

Man Years:
Total: 1½
Professional: 3/4
Other: 3/4

Project Description:

Objectives: To establish a primary avian liver cell culture system in order to further elucidate the mechanism of estradiol induction of the egg yolk phosphoprotein, vitellogenin.

Methods Employed: Several techniques have been used to prepare and culture chicken liver cells. All initially involve the in vivo perfussion of the liver prior to enzymatic digestion with either trypsin or a combination of collagenase and hydronidase. Cells prepared by these techniques have been grown in a variety of culture media, while attached to petri dishes or maintained in spinner flasks. Cells from chickens ranging in age from 15 day-old embryos to 6 week-old cockrels have been prepared.

Initial experiments designed to measure the induction of vitellogenin synthesis in these cells have employed two different assay procedures. In one, the incorporation of 32p-labeled phosphate into the phosvitin moiety of vitellogenin has been measured (ref. Goldstein and Hasty, JBC 248, 6300-6307 (1973)). In the other, immunoelectrophoresis has been used to detect the interaction of antibodies, prepared against purified vitellogenin or lipovitellin, with the protein products secreted by the cells (ref. personal communication Drs. Grieninger and Granick, Rockefeller University).

Major Findings: Chicken liver cells have been prepared and maintained in culture for a period of up to 7 days. During this time the cells appear, morphologically and biochemically, to remain differentiated hepatocytes.

Cells prepared from 15 day-old chicken embryo livers, synthesis the phosphoprotein, vitellogenin, which can be precipitated with antibody prepared against lipovitellin. Very preliminary results of experiments with cells prepared from immature chickens (4 weeks old) suggest that they, too, can be induced to synthesis vitellogenin.

Significance to cancer research: The mechanism by which the expression of particular genes is regulated remains, in large part, unclear. We hope that study of the molecular mechanism of gene expression in this system will provide a better understanding of the role of steroid hormones in such regulation.

Proposed course of research: Further experiments will be conducted to establish the best procedures for preparation and growth of chicken liver cells in culture. Parameters such as the age of the chicken, the length of enzymatic digestion during preparation, the composition of the culture media, etc. will be optimized.

Using the cultured cells, we will study various aspects of the primary and secondary effects of estrogen stimulation. We will be able to determine the type of liver cell capable of synthesizing vitellogenin, to study the nature of the estrogen receptor in these cells, to define more clearly the rates of synthesis and secretion of vitellogenin and to begin to identify more clearly the nature of the changes that occur when vitellogenin synthesis is induced.

Keyword Descriptors: vitellogenin/ avian liver/ estrogen.

Z01 CBD 5231-02 LB
1. Laboratory of Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Subunit Interactions in Enzyme Chemistry

Previous Serial Number: Same

Principal Investigator: Dr. Claude B. Klee

Other Investigators: Dr. Jonathan Craine, Dr. Elizabeth Barbehenn

Cooperating Units: None

Man Years:

Total:	3.2
Professional:	2.8
Others:	0.4

Project Description:

Objectives: To study the role of subunits or protein-protein interactions in two enzymatic systems: polynucleotide phosphorylase from M. luteus and cAMP phosphodiesterase.

Methods Employed: The project involves the study of properties of proteins, purified by the usual techniques of protein isolation, including many types of chromatography. The enzymes are being studied by examining their optical, hydrodynamic, and kinetic properties as well as by suitable chemical measurements and modifications.

Major Findings:
1) Polynucleotide phosphorylase. A new rapid purification procedure using affinity chromatography on poly U sepharose has been developed. It results in high yields of pure, primer independent, enzyme. Under denaturing conditions (SDS gel electrophoresis), the enzyme is dissociated to a single polypeptide chain of 95± 3000 molecular weight. However, under non-denaturing conditions the enzyme exhibits multiple active species. Interactions between the subunits are believed to be responsible for this phenomenon as well as some changes in catalytic properties and are being investigated further. Upon limited treatment with trypsin, the enzyme subunit molecular weight is decreased to 65.000 and the polymerization reaction becomes dependent on the addition of a primer. Furthermore, no multiple comformers are observed (C. Klee, J. Craine, E. Barbehenn).

2) Regulation of cAMP phosphodiesterase. A new highly sensitive and specific assay procedure has been developed which measures the product (AMP) directly,

without conversion to adenosine by 5'-nucleotidase. This assay has been used for the purification of brain soluble cAMP phosphodiesterase and its activator protein. (C. Klee)

Significance to biomedical research and the program of the Institute: Both proteins studied are taken as example of regulation of catalytic activity by protein-protein interactions. Polynucleotide phosphorylase, a well-known bacterial enzyme, also has been described in mammalian cells where its phosphorolytic activity alone has been observed. It is widely utilized for the synthesis of model nucleic acids used in nucleic acid enzymology and interferon induction, and also in the degradation of poly A sequences of mammalian mRNA.

cAMP phosphodiesterase, as one of the enzymes responsible for the control of cAMP levels which are critical for the regulation of cell growth and differentiation, is of considerable interest. Its regulation by endogenous proteins and other factors is undoubtedly of great importance to the cell. The molecular features of this regulation need to be elucidated.

Proposed course of research. Studies on polynucleotide phosphorylase will be directed towards the elucidation of the nature of subunit interactions leading to the multiple enzymatic species. The loss of primer independence upon trypsin treatment will be used as a tool to study the mechanism of initiation of polynucleotide synthesis catalyzed by the enzyme.

Purification and characterization of the multiple forms of cAMP phosphodiesterase (particulate and soluble, low and high affinity forms) will be carried out in order to study the regulation of the enzyme activity by its natural protein activator and low molecular weight ligands.

Publications:

1. Klee, C.B.: Stereospecific irreversible inhibition of histidine ammonia-lyase by L-cysteine. Biochemistry 13: 4501, 1974.

2. Klee, C.B., Kirk, K.L., Cohen, L.A., and McPhie, P.: Histidine ammonia-lyase: the use of 4-fluorohistidine in identification of the rate-determining step. J. Biol. Chem. In press, 1975.

Keyword Descriptors: polynucleotide phosphorylase/ cAMP phosphodiesterase/ protein-protein interaction/ mechanism of enzyme action/ enzyme regulation.

Z01 CBD 5232-02 LB
1. Laboratory of Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Replication of Simian Virus 40 (SV40) DNA in Monkey Kidney Cells

Principal Investigator: Donald J. LeBlanc, Ph.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	1.2
Other:	0.2

Project Description:

Objective: To develop an *in vitro* replication system which closely approximates the *in vivo* situation and to utilize the *in vitro* replication system to study the enzymology of SV40 DNA synthesis.

Methods Employed: Standard tissue culture procedures for the growth of monkey kidney cells and SV40. Adaptation of published procedures for the isolation of cell nuclei and nuclear membrane-associated DNA. Procedures used for studying viral DNA synthesis included radioisotope tracer techniques, preparative and analytical ultracentrifugation, DNA-DNA hybridization and selective precipitation of high molecular weight DNA.

Major Findings: Data obtained with a semi-*in vitro* system, involving nuclei from infected cells, suggest that the replication of SV40 DNA proceeds via a discontinuous mechanism along one strand, while elongation along the other strand occurs in a continuous fashion. The end-product of replication by infected nuclei, in the absence of cytoplasmic factors, appears to be a late replicative intermediate seldom observed in *in vivo* studies. Such a replicative intermediate is characterized by the presence of many small, unlinked, Okazaki-like fragments. Preliminary data suggest that the linkage of these fragments may involve nuclear activities requiring a high concentration of thymidine triphosphate. Further studies have suggested that the total maturation of SV40 replicative intermediates to component I DNA requires factors supplied by the cytoplasm, and that the process is stimulated by the presence of histones.

Significance to Cancer Research: Simian Virus 40 is an oncogenic DNA virus known to produce tumors in hamsters. Experimental evidence shedding light on the mode of replication of this virus is of obvious significance to NCI research.

Proposed Course of Research: More definitive evidence for the proposal that elongation of newly synthesized strands of SV40 DNA involves a continuous, as well as a discontinuous, mechanism is being sought by the utilization of DNA reannealing techniques. The nature of the gap-filling steps required to complete the discontinuous elongation process is also currently being investigated. In addition, attempts to fractionate cytoplasmic factors required for maturation of replicative intermediates to complete component I molecules will be made. Finally, the function of histones in the replication process will also be investigated.

Publications:

1. LeBlanc, D.J. and Singer, M.F. Localization of replicating DNA of Simian Virus 40 in Monkey Kidney Cells. PNAS 71: 2236, 1974.

2. Le Blanc, D.J. and Singer, M.F. Factors affecting circularization of SV40 DNA during in vitro replication by infected nuclei. Abstracts of the Annual Meeting of the American Society for Microbiology. P. 221, 1975.

Keyword Descriptors: SV40/ in vitro replication/ DNA synthesis/ maturation of SV40 DNA/ cytoplasmic requirements for SV40 replication.

Z01 CBD 5234-02 LB
1. Laboratory of Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Replication and Properties of Defective-Substituted Simian Virus 40 in Permissive Monkey Kidney Cells.

Previous Serial No.: Same

Principal Investigators: G. R. Koteswara Rao, Ph.D., Shoshana Segal, Ph.D., Francesco Blasi, M.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years

Total:	2.9
Professional:	2.7
Other:	0.2

Project Description:

Objectives: Serial, undiluted passage of Simian Virus 40 (SV40) yields defective virions containing DNA which is 1) shorter than normal 2) partially substituted with host sequences. We wish to study the preferential replication of such defective-substituted DNA as well as the nature of the host DNA sequences incorporated into SV40.

Methods Employed: Standard tissue culture procedures are used for the growth of monkey kidney cells and SV40. Procedures used for studying viral DNA synthesis included radioisotope tracer techniques, preparative and analytical ultracentrifugation, DNA-DNA hybridization, selective precipitation of high molecular weight DNA, polyacrylamide and agarose gel electrophoresis, degradation with specific restriction endonucleases. Radioisotope tracer methods are essential.

Major Findings: In order to study replication of defective substituted SV40, attempts were made to use one type of defective virus particles. First, a viral clone containing substituted genome was isolated and serial undiluted passages were continued in monkey kidney cells to enrich defective virions. Viral DNAs were prepared from cells infected with passaged cloned virus. In spite of the cloning, with continuing passages the size of viral DNA becomes heterogeneous and smaller as measured by sedimentation on alkaline sucrose gradients and electrophoresis through agarose gel. As described in last year's report, with continuing passages the percent of host sequences in defective viral DNA increased and at the same time the number of plaque forming units in the passage yields decreased. However, at sixth passage, the defective virus changed markedly--the yield of plaque forming units increased by about 1-2 orders of magnitude, the passage gives, upon infection of cells, a rapid cytopathological effect, and rapid viral DNA synthesis.

Studies with Escherichia coli R1 restriction endonuclease (Eco RI) (cleaves non-defective SV40 DNA at only one site) showed with continuing passage that the defective viral DNA became partly resistant to the enzyme cleavage. Studies with Haemophilus influenzae restriction endonuclease showed that the fragments produced by the enzyme cleavage are completely different from those obtained with standard non-defective viral DNA. Further, a fragment of 50,000 molecular weight which hybridizes exclusively to host DNA appears in early passages (passages 2 and 3) of the cloned defective and is absent in late passages (passages 4 through 8). In passage 3 DNA, the repetitive host sequences are present exclusively in Eco R1 resistant fraction of DNA which also contains the 50,000 molecular weight host segment generated with the Haemophilus enzyme. The R1 sensitive fraction lacks both. Studies with Haemophilus aprophilus restriction endonuclease (Hap II) which cleaves non-defective viral DNA at one site showed that Eco R1 resistant passage 3 DNA is cleaved at multiple sites generating approximately one third size fragments.

Thus, these studies demonstrate that even starting with a cloned defective, multiple species of defective virus are present at each passage, and further, that the structure of the DNA of the defectives changes upon each high multiplicity passage. The experiments with the restriction enzymes, coupled with the physical separation methods, have allowed us to purify the various defective molecules present in a given mixture and to begin to elucidate the structure of each one.

We have, in addition, undertaken the purification of the fragment of 50,000 daltons present in the third passage of the cloned defective. This fragment was also present in the mixture of defectives from which the clone was originally derived. The sequence of the nucleotide bases in this fragment, which represents a sequence from the repetitive DNA of the host is being determined.

Significance to Cancer Research: Simian Virus 40 is an oncogenic DNA virus known to produce tumors in hamsters. SV40 and related viruses have recently been identified in association with several human diseases, including a few tumors. Thus, any experimental evidence shedding light on the mode of replication of this virus is of obvious significance to NCI research. Further studies on substituted defective SV40 have 3 broad implications:

First, they raise the possibility of transduction experiments in animal systems, and second, the formation of the substituted molecules provides an interesting approach to the metabolism of DNA in particular, recombination.

Third, defective animal virions occur upon passage of many viruses, and it has been proposed that they play a part in the self-limitation of viral infections.

Proposed Course of Research: We will try to investigate the mechanism whereby defective viral DNA is synthesized preferentially over non-defective DNA. Elucidation of the structure of the defective DNAs should aid this study. Further, we are interested in understanding how substituted molecules arise. This will entail investigation of the nature of those host sequences which are incorporated into substituted SV40 DNA. Detailed study of the 50,000 dalton sequence of repetitive DNA should permit us to localize at least one point of recombination between viral and host DNA. We shall also carry out studies on the mechanism by which defective SV40 inhibits the replication of standard, non-defective SV40.

Keyword Descriptors: Simian Virus 40/ defective substituted virus/ DNA synthesis/ host sequences.

Z01 CBD 5237-02 LB
1. Laboratory of Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Replication of Simian Virus 40 DNA in Monkey Kidney Cells

Previous Serial No.: Same

Principal Investigator: Tikva Vogel, Ph.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years

Total: 1.6
Professional: 1.4
Other: 0.2

Project Description:

Objectives:

1. To study the DNases associated with the conversion of the closed supercoiled form of SV40 DNA into the nicked relaxed form. This reaction is proposed to be required for initiation of viral DNA replication and recombination.

2. To study the interaction between mammalian histones and DNA in vitro and in vivo, with particular emphasis on SV40 DNA and other superhelical DNA.

Methods Employed: Standard tissue culture techniques for growth of monkey kidney cells and SV40 (Simian Virus 40) DNA. Subcellular fractionation, column chromatography, radioisotopes tracer techniques, gel electrophoresis, preparative and analytical ultracentrifugation, millipore binding technique, standard protein purification. Enzymic techniques for preparation of circular DNAs of known superhelicity.

Major Findings: The interaction of superhelical, circular DNA with purified calf thymus histone fractions was studied. The primary technique utilized involved a method depending on nitrocellulose filters. Similar methods have been used previously by other workers to determine the binding of DNA to a variety of proteins. The method depends on the fact that proteins bind to nitrocellulose, and if the protein binds a DNA molecule, the DNA will also bind to the filter by virtue of the binding of the protein. If the DNA is radioactively labeled, as it was in our experiments, it is relatively simple to determine the binding of the DNA to the filter as a function of the presence of the protein, in this case, the histones. In the absence of a protein to which the DNA binds, no radioactivity is absorbed to the filter and the DNA passes right through.

We found that five to ten fold less f1 histone is required to complex a given amount of superhelical DNA compared to the other four common histone fractions. When the superhelical DNA is converted to either the relaxed circular form, or full length linear molecules, or fragmented linear or denatured strands, the efficiency of complex formation with f1 histone' markedly decreases. We concluded from these experiments that f1 histone has a special ability to interact with superhelical DNA. Experiments were carried out with several different superhelical DNAs with similar results.

Furthermore, the interaction of f1 histone with a series of superhelical molecules with varying degrees of superhelicity was also studied. The series of molecules was prepared by addition of varying amounts of the intercalating dye, ethidium, to the DNA. As has been shown by other workers, depending on the amount of ethidium bound to a closed circular DNA molecule, the degree of superhelicity can be varied from a negative superhelicity, through zero superhelicity and with more ethidium, to positive superhelical values. We found that the extent of interaction of f1 histone increases with increasing superhelicity regardless of whether the superhelicity is of the positive or negative sense. In addition, we demonstrated that the interaction of f1 histone with superhelical DNA is readily reversible.

In the several proposed structures for mammalian chromatin recently published, no specific structural role is assigned to f1 histone. The structures involve exclusively the other four main histone fractions. This is because the basic structure of chromatin revealed by X-ray diffraction techniques does not depend on the presence of f1 histone. In the mid 1960s, Mirsky and co-workers proposed that f1 histone is involved in the cross-linking or condensation of chromatin fibrils. If, as suggested by our data, f1 histone interacts preferentially with twisted double-helices, that is, with four-stranded structures, then such twisted configurations, stabilized by f1 histone, may be involved in the folding of DNA double helices in the regions between the regularly spaced complexes of the other four histones.

Significance to Cancer Research: The control of genetic information in eukaryote cells is a primary question in any consideration of oncogenic processes. The role of histones in such controls is a matter of intensive current research and speculation in many laboratories. Our findings represent a completely new observation about the possible function of one of the histones in this control.

Proposed Course of Research:

1) Characterize the nature of the f1 histone-superhelical DNA complexes by means of centrifugation, electron microscopy, and circular dichroism.

2) Determine the stoichiometric relation between the f1 histone and the DNA using radioactivly labeled histone, which we currently have in hand.

3) Study the structural features of the f1 histone molecule which are involved in the reaction specificity. We intend to study the role of histone phosphorylation in the reaction since there are several reports in the literature suggesting a relation between f1 phosphorylation and the condensation of chromatin during mitosis. We would also like to use published procedures for the isolation of well characterized portions of the f1 molecules to study what part of the molecule is essential for the specific interaction with superhelical DNA.

4) We have already initiated preliminary studies on the synthesis of histone concomitant with the synthesis of SV40 DNA in infected monkey kidney cells. We would like to study the synthesis of f1 histone in order to obtain data on the role of f1 histone in the metabolism of superhelical DNA.

Publication:

Vogel, T. and Singer, M.: The Interaction of Histones with Simian Virus Supercoiled Circular Deoxyribonucleic Acid *in vitro*. J. Biol. Chem. 250: 796-798, 1975.

Keyword Descriptors: SV40/ DNase/ histones/ superhelices/ *in vitro* interaction of f1 histone with SV40 DNA.

Z01 CBD 333-12 LB
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The biochemical basis for defective maturation in myelogenous leukemia

Previous Serial No.: Same

Principal Investigator: Warren H. Evans

Other Investigators: Elbert A. Peterson, Michael Mage, Shirley Wilson, Rani Rao, Charles K. Grieshaber

Cooperating Units: Laboratory of Biology, DCBD, NCI

Man Years:
Total: 4
Professional: 4
Other: 0

Project Description:

Objectives: 1) To identify the main molecular constituents and metabolic processes which distinguish mature granulocytes from their immature precursors. 2) To determine whether the major structural and biochemical changes associated with granulocyte maturation are regulated by factors present in body fluids and/or by cell-mediated factors. 3) To use the information gained from (1) and (2) to; (a) identify the primary molecular abnormalities associated with the arrested maturation of granulocytes in acute and chronic myelogenous leukemia and possibly develop methods for the early diagnosis of leukemia, (b) develop specific biological and/or biochemical methods for inducing leukemic cells to develop some or all of the functional characteristics of mature granulocytes as a means of partial or complete restoration of host defense mechanisms in leukemia patients.

Methods Employed: Leukocytes are isolated from bone marrow, blood, and inflammatory exudates by a variety of methods. Subcellular fractions are prepared by differential and zonal centrifugation and further fractionated by chromatography and electrophoresis. Immunological reagents, including fluorescent antibodies, are prepared from the purified fractions and used to follow the development of related material during maturation of the cells. The maturation of enriched cell populations is studied in soft agar and stationary tissue culture. The incorporation of radioactive precursors into proteins and nucleic acids of cells is studied, using liquid scintillation.

Major Findings:

1. Protein Profiles of Granule Proteins in Mature and Immature Granulocytes. The post-nuclear supernatant fraction of sucrose homogenates of mature granulocytes was subjected to differential centrifugation to obtain a total particulate fraction, a particle-free supernatant fraction, highly enriched fractions of primary and secondary granules, and a membrane-rich fraction. Enzymatic markers and electron microscopy were used to determine the degree of enrichment of the various fractions. Each of these fractions was solubilized in buffer containing sodium dodecyl sulfate (SDS) and analyzed for protein and glycoprotein component by SDS-polyacrylamide gel electrophoresis. The major glycoprotein component of the post-nuclear supernatant fraction were found mainly associated with the enriched fraction of secondary granules and, to a lesser extent, with the membrane rich fraction. No major glycoprotein components were visible in the polypeptide electrophoretic patterns of the primary granule fraction or of the particle-free supernatant fluid.

Attempts at separation of leukocyte granules by zonal sucrose density gradient centrifugation were only partially successful. The data indicate a species difference in this regard between rabbit and guinea pig leukocyte granules.

2. Role of Arginase in Granulocyte Maturation. Our studies show that mature granulocytes in bone marrow lack the enzyme ornithine transcarbamylase, suggesting that the urea cycle is absent in these cells in spite of the presence of arginase, a key enzyme in this cycle. We also have obtained evidence that during the maturation of granulocytes in the bone marrow, arginase, an ornithine-producing enzyme, develops asynchronously in relation to the ornithine-utilizing enzymes ornithine aminotransferase and ornithine decarboxylase.

3. Nuclear Changes in Maturing Granulocytes. Techniques for preparing nuclei from leukocytes involving sucrose homogenization have been unsatisfactory since most nuclei are disrupted by this process. We have developed a method involving gentle homogenization of leukocytes in a citrate buffer which yields intact nuclei essentially free of cytoplasm. This method is proving useful in studies on the histone content of mature and immature leukocyte nuclei.

Significance to Cancer Research: (Objective 2, Approach 3) Understanding of normal and leukemic granulocyte maturation depends upon clarification of the molecular processes whereby the cell controls the appearance and disappearance of specific components. Such information has never been obtained at the biochemical level in a systematic fashion similar to that achieved in morphological studies of normal and leukemic granulocytes. Any biochemical differences in the maturation processes observed between normal and leukemic leukocytes will be exploited for purposes of developing techniques, presently unavailable, for the early diagnosis of leukemia and for improved treatment, especially with regard to the possibility of reversing the arrested maturation and decreased phagocytic activity of leukocytes from myelogenous leukemia.

Proposed Course of Research: The formation of primary and secondary granules in immature granulocytes is one of the prime indicators of differentiation in these cells. The factors which regulate the synthesis of specific proteins in these granules are still unknown. We will attempt to develop a biochemical assay for these factors, using C^{14} amino acid incorporation into granule proteins as an indicator of cell differentiation. This approach will require the development of methods for purifying granules from immature granulocytes and methods for completely extracting their major protein components. Using polyacrylamide gel electrophoresis it should be possible to screen the major protein components for their relative contents of radioactive amino acids. The effect of putative regulatory factors on the incorporation of amino acids into these proteins could then be determined.

Studies are underway to further clarify the role of arginase, ornithine decarboxylase, and ornithine aminotransferase in the metabolism of mature and immature granulocytes. Since our studies indicate that mature cells lack the enzymes necessary for the further metabolism of ornithine produced by arginase, we will investigate the possibility that ornithine is transferred outside the cell. Such a transport system, if present, would have important implications for the feedback regulation of the metabolism of immature granulocytes, which appear to lack arginase but contain ornithine-utilizing enzymes.

Studies on nuclei from mature and immature granulocytes will be aimed at determining the significance of nuclear folding in granulocyte development. The functional capacity of the DNA in these nuclei to serve as templates for RNA synthesis will be studied. If RNA synthesis occurs in mature granulocytes, it should be possible to localize subcellularly those proteins whose synthesis is dependent on new RNA synthesis. This information might give us a clue to the function of such proteins.

Honors and Awards: None

Publications:

1. Evans, W.H., Wolf, M.M., and Chabner, B.A.: Concentration of Immature and Mature Granulocytes From Normal Human Bone Marrow. Proc. Exp. Biol. Med. 146: 526-529, 1974.

2. Noseworthy, J., Smith, G., Himmelhoch, S.R., and Evans, W.H.: Protein and Glycoprotein Electrophoretic Patterns of Enriched Fractions of Primary and Secondardy Granules From Guinea Pig Polymorphonuclear Leukocytes. J. Cell Biol.: (In Press, 1975.

Keyword Descriptors: bone marrow/ leukocytes/ granulocytes/ differentiation/ leukemia/ regulation/ cell separation/ maturation/ enzymes/ glycoproteins.

Z01 CBD 375-13 LB
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Homogeneity and structure of proteins

Previous Serial No.: Same

Principal Investigator: Elbert A. Peterson

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1 2/3
Professional: 2/3
Other: 1

Project Description:

Objectives: To develop methods for the separation and analysis of proteins that will overcome present limitations and to apply these methods to the purification of specific proteins for the study of their function and structure.

Methods Employed: A variety of polyanions and polycations differing in molecular size, charge density, and chemical composition are prepared by appropriate reactions. Most of these are ionic derivatives of low molecular weight dextrans but other types are being explored. They are evaluated as displacing agents, using test protein mixtures, on hydrophilic ion exchangers. Effluent fractions are examined by electrophoresis in slabs of polyacrylamide gel and by appropriate assay.

Major Findings: Significant concentrations of salt displaced as counter-ions from the ion exchanger during development of the displacement chromatogram have, in past work, interfered with the adsorption of low-affinity proteins, necessitating the use of special procedures to prevent loss of resolution when such proteins were to be part of the scan. The use of DEAE-agarose, a recently introduced commercially available ion exchanger, has essentially eliminated this problem because of its low, uniform density of charged groups.

Substantial improvements have been made in the synthesis and use of CM-dextran mixtures that provide smoothly rising affinity continuums, with corresponding improvement in resolution. Even at the present incomplete state of development, The combined displacement chromatography and polyacrylamide gel electrophoresis procedure is capable of detecting many more of the minor proteins of human serum than can be resolved by either alone, and the bulk of each chromatographic fraction thus characterized remains available for additional steps in the isolation of whatever components are of interest.

Significance to Cancer Research: (Objective 2, Approach 3). Displacement chromatography of proteins, nucleic acids, etc. promises to be of considerable value at any scale of operation and therefore has significance to all research that involves the isolation of such substances. Its immediate significance to the program of this Section, however, lies in its potential application to the fractionation of the small quantities of protein available in each fraction obtained when bone marrow cells are separated into populations of different cell types. The displacers, unlike salt, do not interfere with electrophoresis, and protein fractions obtained by displacement can be directly subjected to electrophoresis or rechromatography without dialysis or concentration. Thus the examination of complex mixtures by a combination of chromatography and electrophoresis on a semi-micro scale is greatly expedited. The simplicity of operation also facilitates automation, so that the two-dimensional analysis of large numbers of fractions becomes feasible.

Moreover, since migration is induced by displacers under conditions of salt and pH in which the migrating molecules are otherwise strongly adsorbed, the possibility arises of chromatographing substances that are "irreversibly" adsorbed in conventional procedures. Also, theoretical considerations strongly suggest that many of the enzymes that become labile when purified can be protected from inactivation or loss by substances such as the CM-dextrans if the metal-sequestering properties of the system are taken into account.

Proposed Course of Research: The method, as it develops, will be applied to the examination of proteins and nucleic acids in guinea pig bone marrow cells. Its possible application in the fractionation of cell populations and subcellular organelles will be explored, using appropriate adsorbent matrices. Also, a stabilizing effect of the CM-dextrans will be looked for, using enzymes that have previously been difficult to purify because of their high lability.

Honors and Awards: None

Publications: None

Keyword Descriptors: chromatography/ displacement/ fractionation/ gel
electrophoresis/ homogeneity/ ion exhange/ poly-
electrolytes/ proteins/ purification.

Z01 CBD 5202-08 LB
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation, fractionation, and characterization of native nucleoproteins

Previous Serial No.: Same

Principal Investigator: O. Wesley McBride

Other Investigators: John B. Burch, Raghbir S. Athwal, Miles C. Otey, Elbert A. Peterson

Cooperating Units: None

Man Years:
Total: 5
Professional: 3
Other: 2

Project Description:

Objectives: The determination of the role of nucleic acid-bound proteins in the regulation of the transcription and translation of genetic information, and the biological transformation of cells with isolated and fractionated mammalian metaphase chromosomes as a means of genetic mapping.

Methods Employed: Tissue culture procedures involving propagation of mammalian cells in monolayer and suspension cultures, plating in selective media, cloning, labelling with radioisotopes, selection of cell mutants, and metaphase arrest for chromosome isolation. Metaphase chromosome isolation and purification by isopycnic and velocity sedimentation (including unit gravity procedures), and evaluation of chromosomal DNA molecular weight by zonal density gradient sedimentation. Gene transfer to mammalian cells via uptake of purified metaphase chromosomes and isolation of biologically transformed colonies in selective media. Isolation, quantitation, and characterization of gene products by chromatography, electrophoresis, isoelectric focusing, and immunochemical procedures.

Major Findings: The stable transfer of x-linked gene, HPRT, to mouse cells, has been demonstrated following incubation of purified metaphase chromosomes, isolated from diploid human cells, with mouse A9 cells and isolation of clones in selective HAAT medium. The HPRT gene product from 4 independent clones was characterized as human species by both starch gel electrophoresis and thin layer isoelectric focusing in polyacrylamide gels. However, the human species of 2 other x-linked enzymes, G6PD and PGK, was not found in any of these 4 clones. These results indicate that only a portion of the x chromosome was

was expressed after transfer; probably part of the chromosome is lost during uptake due to lypsosomal degradation. The absence of a marker (PGK) proximal to hprt, as well as one distal (G6PD) to it on the long arm of the x-chromosome, suggests that the transferred hprt gene is probably integrated into the recipient mouse DNA in these clones.

Colonies have been isolated in selective HAAT medium after incubation of TK-deficient B_{82} mouse cells with metaphase chromosomes isolated from wild type chinese hamster fibroblasts. Two of 11 clones also contain the chromosomal donor species of galactokinase, indicating that closely linked genes are sometimes transferred together in this procedure. The clones were examined for the chromosomal donor species of 9 non-linked enzymes and none were detected; thus these colonies did not arise through cell fusion. Reversion studies indicate that a large fraction of these 11 colonies are true "transformants" and not revertants. However, stability of the transferred gene in these colonies varies over a wide spectrum from less than 1% loss over the course of 60 cell generations to $\geq$ 50% loss over this same interval. The electrophoretic system of Kit has been modified to provide autoradiographic detection of cytoplasmic thymidine kinases after slab polyacrylamide gel electrophoresis; the human species is readily distinguished from hamster or mouse species but the latter two are not adequately resolved.

A system has been developed for 200-fold purification of ch. hamster APRT by affinity chromatography in high yield (>50%) and with great stabilization. The affinity adsorbent is prepared by covalent linkage of the enzyme product and competitive inhibitor, 5'-AMP, to aminohexyl agarose through the 2', 3' ribose carbons after periodate oxidation. Non-specific proteins and HPRT are eluted from this adsorbent in the presence of 0.5 or 1M KCL, whereas APRT is retained and it is specifically eluted by the substrate Mg PRPP (10 mM) in the presence of 1M KCL. The enzyme HPRT binds to the analogous affinity adsorbent, IMP-aminol-exyl-agarose, but it cannot be eluted from this adsorbent.

Several modified Sephadex, ion-exchange adsorbents have been prepared and evaluated for metaphase chromosome fractionation. An adsorbent with a very high binding capacity for chromosomes which permits elution of all chromosomes under non-denaturing conditions has not been developed yet. Partial resolution of Ch. hamster chromosomes into different size classes has been obtained by unit gravity sedimentation.

Proposed Course of Research: Clones resulting from transfer of human hprt to mouse cells are under evaluation for the presence of another x-linked enzyme, α-galactosidase. Efforts will also be made to detect human chromosome fragments by karyological methods and hybridization studies. Chloramphenicol (CAP) resistant mutants of all these clones will be isolated; after enucleation, the location of the transferred human gene in these cells will be determined by fusion of resultant nuclei and enucleated cytoplasm, independently, with CAP-sensitive, HPRT-deficient chinese hamster cells. A nuclear location is anticipated, of course, but it is now important to verify this assumption. The putative linkage of thymidine kinase and galactokinase in clones resulting from chromosome transfer will be examined by analysis of segregants. Chromatographic and electrophoretic methods are being developed to distinguish the two rodent species of cytoplasmic TK; the two galactokinase species currently can readily be distinguished electrophoretically.

The purification of APRT by affinity chromatography will be evaluated further by characterization of the purified protein by multiple criteria including non-denaturing and SDS polyacrylamide gel electrophoresis. The general utility of this approach will be verified by affinity chromotography of APRT from other species also. Affinity adsorbents to purify HPRT will be prepared and tested.

Additional methods for fractionation of metaphase chromosomes will be investigated. Modified systems for chromosome transfer will be explored to provide expression of an intact transferred chromosome. Non-reverting mutants with simple karyotypes are being isolated to simplify kanyological analysis.

Significance to Cancer Research: (Objective 2, Approach 3) Metaphase chromosome isolation and fractionation provides the most promising approach for the fractionation of mammalian genes and can provide material for the investigation of control elements associated with specific sets of genes. The biological expression of these chromosomes introduced into recipient cells provides a method for mapping of mammalian genes, investigating differences between genetic elements in normal and neoplastic cells, and looking for sites of integration of oncogenic viruses into the mammalian genome.

Honors and Awards: None

Publications:

1. McBride, O.W.: Metaphase Chromosome Uptake By Mammalian Cells And Expression of the Gene Transferred. in Stadler Symposium 4: 23-46, 1974. (Ed.) G. Redéi)

2. Burch, J.B., and McBride, O.W.: Human Gene Expression in Rodent Cells After Uptake of Isolated Metaphase Chromosomes. Proc. Nat. Acad. Sci. USA, May 1975.

Keyword Descriptors: metaphase chromosomes/ gene transfer/ gene mapping/
gene linkage/ HPRT/ APRT.

Z01 CBD 5203-07 LB
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of immunochemical and physical methods for the purification and characterization of immunocytes and their subcellular components

Previous Serial No.: Same

Principal Investigator: Michael G. Mage

Other Investigators: Louise McHugh

Cooperating Units: None

Man Years:
Total: 1½
Professional: 1½
Other: 0

Project Description:

Objectives: The goal of this project is the development of methods for the specific isolation of immune cells, particularly for antigen-reactive cells involved in cellular immune reactions to alloantigens, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules.

Methods Employed: Antigen-reactive cells (ARC) are obtained from the spleens of normal mice or from mice immunized with allogeneic tumor cells and normal cells. Spleen cell suspensions containing ARC are incubated on foam columns or on petri plates coated with allogeneic or syngeneic target cells. Graft-versus-host (GVH) activity of nonadherent spleen cells is measured by the Simonsen spleen weight assay in neonatal F_1 mice. Cytotoxic activity is measured by ^{51}Cr release from target cells.

Major Findings: We have previously found that when spleen cells are incubated on allogeneic target cell monolayers, cytotoxic effector cells are bound to the monolayers but GVH-ARC are not. We have now found that when spleen cells are incubated and then briefly centrifuged on allogeneic monolayers, some of the GVH-ARC are bound, as evidenced by depletion of about 50% of the GVH activity (directed against alloantigens present on the monolayer cells) from the nonadherent cell population. The specificity of the depletion is shown by the failure of third-party allogeneic monolayers to remove GVH activity, and by full activity on the part of the nonadherent population, on injection into third-party neonates. Following up on this finding, we are attempting to delineate which specific antigens need to be present on the target cell monolayers for binding of GVH-ARC to occur. Current experimentation involves using cells from different tissues as target cells for absorption, and the use of foam columns and Sephadex bead columns, as well as petri plates as substrates for the monolayers of target cells.

Significance to Cancer Research: (Objective 2, Approach 1) These studies on the specific separation of antigen-reactive cells relate to the mechanisms of cell-mediated tumor rejection. Knowledge of the antigen-binding characteristics of immune cells may help in development of specific purifications of immune lymphocytes with antitumor reactivities and may aid in understanding the variation in tumor susceptibility of human beings and make possible control of immune mechanisms of tumor surveillance and rejection.

The GVH reaction is an iatrogenic complication of tumor therapy regimens that include bone-marrow transplantation. A specific separation of GVH-antigen reactive cells might aid in avoiding this complication.

Our reason for working with antigen-coated foam columns (which we have used to specifically bind antibody-producing cells) is that these columns have high binding capacity (on the order of 10^8 cells/cc) and thus have the potential for preparative scale separations of immune lymphocytes.

Proposed Course of Research: As an aid to the development of general methods for the specific isolation of antigen-reactive lymphocytes, we plan to further study the antigen-binding characteristics of immune T-cells, particularly with respect to target-cell antigen specificity, and conditions needed for antigen-binding by ARC, and the conditions needed for the stable binding of lymphocytes to insoluble substrates.

Honors and Awards: None

Publications: None

Keyword Descriptors: graft-vs-host/ immune cell separation/ histocompatability antigens.

Z01 CBD 5230-02 LB
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical properties of neoplastic cell membranes

Previous Serial No.: Same

Principal Investigator: Dante Marciani

Other Investigators: John P. Bader, Elbert A. Peterson, Takumi Okasaki

Cooperating Units: Chemistry Branch, DCCP, NCI

Man Years:
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: The study of the modifications of membrane components, mainly proteins that occur in cells transformed by a temperature-sensitive mutant of Rous Sarcoma virus: the study of the role of these modifications in properties of the cell that are expressed at, or mediated by, the cell surface.

Methods Employed: Isolation of cell membranes by differential and density gradient centrifugation. Analysis of the membrane composition by electrophoretic and chromatographic techniques. Study of the surface topography by use of enzymic labeling with ^{125}I or tritium, combined with electrophoresis. Determination of receptors for radioactively labeled lectins.

Major Findings: Studies during the past year were concerned primarily with plasma membranes from chicken fibroblasts, normal and transformed by a temperature-sensitive mutant (Ta) from Rous sarcoma virus isolated by Dr. J. Bader.

(1) Electrophoretic analysis in polyacrylamide gels containing sodium dodecyl sulfate and urea has shown that cells transformed by Rous sarcoma virus (Bryan) present differences from normal chick fibroblasts. Cell surfaces from transformed cells showed a significant decrease in a 250,000 mol. wt. polypeptide and also a depletion in a 73,000 mol. wt. polypeptide. Cells transformed by RSV-BH-Ta and maintained at the permissive temperature, 37°C, showed electrophoretic patterns similar to those observed with cells transformed by the wild type of RSV-BH. Shifting the cells to 41°C resulted in an increase in the 250,000 mol. wt. polypeptide, although the amount of this polypeptide never reached that found in noninfected cells. Although inhibitors of RNA and protein synthesis failed

to block the morphological changes in RSV-BH-Ta cells after temperature shifts from 37°C to 41°C or vice versa, the same inhibitors caused a reduction in the level of 250,000 mol. wt. polypeptide at both temperatures. These data indicate that this large membrane-associated polypeptide plays little or no role in the morphological changes associated with transformation and its reversal.

(2) Studies of the binding of concanavalin A to the cell surface were carried out using [^{3}H] concanavalin A prepared by acetylation with [^{3}H] acetic anhydride. Chick embryo cells, noninfected or infected with RSV-BH, bind the same amount of con A per cell at 4°C and 25°C. Analysis of the binding data by Scatchard plot showed changes in the thermodynamic properties of the binding process. The population of con A receptors on the cell surfaces of normal and transformed cells showed no significant heterogeneity in their binding properties. Chick embryo cells infected with RSV-BH-Ta showed a binding behavior that was dependent on the temperature at which the cells were maintained. At the permissive temperature for transformation (37°C), the process of con A binding was similar to that observed for normal chick embryo cells and RSV-BH infected cells. At the nonpermissive temperature (41°C), analysis of the data showed at least two sets of con A receptors. A new set of receptors had lower affinities than those observed in the same cells maintained at 37°C.

In agreement with previous reports, RSV-BH infected cells showed enhanced agglutinability by con A. In RSV-BH-Ta infected cells a total reversal of the usual correlation between increased con A agglutinability and transformed state was observed. At the permissive temperature for transformation the cells were not agglutinable, whereas at the nonpermissive temperature they presented agglutinability indexes as high as those observed with RSV-BH infected cells. A new property was expressed at 41°C, namely hemadsorption. This property was temperature dependent; a shift from 41°C to 37°C caused a rapid fall in the hemadsorption capacity.

Proposed Course of Research: We are planning to study the changes in surface topography as a function of temperature shift. These studies will involve isolation and characterization of the nature of lectin receptors.

Study of possible modifications of membrane components, such as phosphorylations and changes in glycoproteins, using radioactive precursors and electrophoretic methods. These changes will be studied as a function of temperature and correlated with other altered properties of transformed cells, such as glucose and water transport.

Significance to Cancer Research: (Objective 3, Approach 5) The fact that the sociology of cells is mediated through their cell surfaces makes the study of cell membranes important for understanding the lack of contact inhibition, the changes in permeability, and the capacity for invading normal tissue that are characteristic of neoplastic cells.

Honors and Awards: None

Publications

Bader, J.P., and Marciani, D.J.: Characteristics of Cells Transformed by Rous Sarcoma Virus. In Gottlieb, A.A., Plescia, O.J., and Bishop, D.H.L. (Eds.): Fundamental Aspects of Neoplasia. Springer, New York (In press).

Keyword Descriptors: study of changes in cell membrane induced by oncogenic viruses.

PHS-NIH
Summary Report
July 1, 1974 through June 30, 1975
Laboratory of Biology
NCI

The work of the laboratory continues to be focused on mammary tumorigenesis and related subjects. Much of the work is carried out on our well defined genetic material in our well characterized inbred strains of mice with more recent attempts to transfer the problems to cell culture of these tissues and of tissues from human sources. Drs. Hoffman and Grieshaber are approaching the problem through biochemistry and biochemical genetics describing genetic control of biochemical differences between the inbred strains, defining and measuring enzymatic and hormonal differences between normal, developing, and neoplastic mammary tissue and between viral infected and noninfected tissues, and determining the gene regulation of these differences to gain more insight into the mechanisms of tumorigenesis. Drs. Smith and Longfellow are interested in the mammary tumor virus--host cell interaction as viewed from morphological, biochemical, and immunological aspects. Considerable emphasis is given to the A particle and its ontologic relationship to the B particle recognized as the mature mammary tumor virus. Dr. Heston has narrowed his efforts to describing the genetic transmission of the mammary tumor virus. Mr. Vlahakis is interested in identifying differences in tumor induction capacity of the various lines of the mammary tumor virus and the susceptibility of the different mouse strains to the various lines of virus. Drs. Stromberg and Speeg are primarily interested in induction of oncornavirus expression by hybridization of mouse cells and extending this animal model system to the induction of human oncornavirus expression by hybridization of mouse and human cells.

The enzyme that has received greatest emphasis in the work of Drs. Hoffman and Grieshaber is catalase. They have shown that specific genetic loci control the activity of this enzyme in specific organs of the mouse. Liver catalase is controlled by the Ce-1 gene as yet not located in the genome. Erythrocyte catalase is controlled by the Cs-1 gene now shown to be on chromosome 2 approximately 26 centi-Morgans from the agouti locus. Isozymes of kidney catalase have been shown to be controlled by a single autosomal locus with two alleles apparently linked with the H-2 complex on chromosome 17.

Dr. Hoffman holds a recognized important position in the mapping of the biochemical markers characterizing the inbred strains of mice. Dr. Grieshaber has much interest in the differentiation of enzyme expression and how this modifies cellular function in different tissues, normal and malignant. He is particularly concerned with polyamine concentration and rate of synthesis and how these relate to appearance of

M MTV antigens, to the change in mammary tumors from hormone dependence to independence, and how inhibitors of polyamine synthesis may effect mammary tumor progression.

Dr. Heston, in collaboration with Dr. Wade Parks who is doing the assay for M MTV antigen, is attempting to ascertain whether or not the mammary tumor virus information in the mouse is transmitted as a single dominant gene as has been hypothesized. The key to answering this question is the analysis of the 2nd backcross from crosses between strains GR and C57BL. Segregation ratios obtained here thus far indicate that there is a major dominant gene controlling antigen expression but there is also evidence that this is not the only gene involved. Also parity is a factor. Evidence thus far indicates that this major dominant gene is not the viral information itself but is a controller gene (the old time susceptibility gene) regulating the expression of the viral information presumably located elsewhere in the host genome possibly not as a specific locus and possibly occurring in all mice. We still must get much more information on expression of antigen in these hybrids and then correlate these results with eventual appearance of mammary tumor. The study is going very well but will require a year and one-half yet for completion.

With his broad knowledge of the inbred strains of mice we have and the occurrence of tumors in them, Mr. Vlahakis continues to serve a real function in guiding younger investigators in getting their studies properly underway with suitable strains and tumors. Furthermore, he continues to broaden his knowledge of the different lines of the mammary tumor virus and how each behaves in the various mouse strain - virus line and gene - virus combinations.

The work of Drs. Smith and Longfellow is aimed at understanding the intracellular events and elucidating the replicative cycle of the mouse mammary tumor virus eventually to specify the tumorigenic influence of the virus. A major finding has been a high molecular DNA unique to the intracytoplasmic A particle that has been thought to give rise to the B particle, the mature MTV an RNA virus. The presence of this DNA in the A particles was confirmed by incorporation of ^{3}H-thymidine (a DNA tracer) in tumor tissue with A particles and observing association of grains with the A particles in the autoradiographs. This A particle DNA has been characterized. RNA was also found in the purified A particles but it was of low molecular weight in contrast with that in M MTV. Earlier SDS-polyacrylamide analyses had shown considerable disparity in molecular sizes of polypeptides of the A and B particles. A study now has been completed showing this disparity to be due to the presence of uncleaved M MTV structural proteins in the A particles.

Dr. Longfellow in collaboration with Drs. Gantt and Smith has been studying the relationship of DNA methylation to DNA replication and neoplasia using controlled paired cell lines of neoplastic and normal mouse fibroblasts. Specific molecular events leading to DNA methylation during replication and their role in the biological function of DNA (possibly neoplasia) were described.

With his background experience with the avian myeloblastosis virus, the techniques of isolating the core from the capsule of the virion, and the characterization of the components, Dr. Stromberg has returned from his two years in the laboratories of Spiegelman and of King and has turned his efforts toward the detection of oncornaviral information in human tissues. He and Dr. Speeg have chosen the trophoblast cell as the most likely place to be able to induce virus expression. The approach is through in vitro cell fusion and chromosome segregation. As an animal model system methods of culture of mouse trophoblast cells have been developed. The capacity of cell fusion and resulting chromosome segregation to induce oncornavirus expression in hybrid clones of the C57BL/6 strain of mice has been established. Oncornavirus presence in culture fluid medium is detected by electron microscopy and assay for reverse transcriptase activity. These methods are now being adapted to human trophoblast tissue.

Concurrently, Dr. Speeg is studying control mechanisms of basic cell functions in normal human trophoblast and human choriocarcinoma cells in culture. Effects of modulating factors such as cyclic nucleotides, steroid and peptide hormones, anti-metabolites and inhibitors on two differentiated functions -- human chorionic gonadotropin hormone and placental alkaline phosphatase are being studied.

Identification No. Z01 CBD 03208-06 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Gene Regulation of Protein Turnover and Its Relationship to Tumorigenesis

Previous Serial No.: Same

Principal Investigator: Harold A. Hoffman

Other Investigators: Charles K. Grieshaber
Warren Evans (Laboratory of Biochemistry, NCI)
Jo Hilgers (Netherlands Cancer Institute)
E. A. Boyse (Sloan Kettering Institute)
S. Nandi (Cancer Research Laboratory, Univ. of California, Berkeley)
M. R. Banerjee (Tumor Biology Laboratory, Univ. of Nebraska)

Cooperating Units: Office of Program Resources and Logistics, DCCP, NCI (Supplied rabbit anti-MTV serum)
Transplantation Immunology Branch, NIAID (Supplied allotypic histocompatibility typing sera)

Man Years:

Total:	5.4
Professional:	2
Others:	3.4

Project Description: Objective 3. Approach 3

Key word descriptors: Regulatory enzymes, biochemical genetics, tissue and erythrocyte catalase, inbred mice

Objectives: To study by methods appropriate to biochemical and quantitative genetics the gene regulation of protein synthesis and degradation in the mouse and other mammals and to determine the genetic and nongenetic relationships between protein metabolism and tumorigenesis. To determine the primary structure of the normal protein and the aberrant or mutant protein in order to define the gene or genes controlling their production and to determine the time of gene action during the process of neoplastic transformation.

Methods: In these investigations mice from 18 inbred strains and substrains, which represent at least six different independent lines of known genetic origin, are used as sources of proteins and tumors, and for the production of allotypic antiserum. New Zealand white rabbits of known immunoglobulin type are used for the production of heterologous antiserum, and non-human primates of the order anthropoid are used as a source of protein.

Immunochemical methods, agar-gel double diffusion, immunoelectrophoresis, and immunofluorescent absorption, are used in the detection and characterization of different proteins. Physical and chemical techniques of gel electrophoresis and chromatography are used to isolate and classify different proteins. The fine structure and minute differences between closely related proteins are determined by peptide map analysis (fingerprints) and amino acid analysis.

Major Findings:

Inheritance of tissue and erythrocyte catalase in inbred mice. Linkage analysis has shown that the loci controlling liver catalase activity (Ce-1) and erythrocyte catalase activity (Cs-1) are genetically independent of each other. The linkage group of Ce-1 is still unknown. The Cs-1 locus, located on chromosome 2, is approximately 26 centi-Morgans from the agouti locus.

Total erythrocyte enzyme activity is an expression of the specific activity of the enzyme and the intracellular concentration. The specific activity and enzyme concentration of RBC catalase for BALB/c, C57BL, C57BL/6 and NBL were measured to determine the mechanisms responsible for the variation in enzyme activity observed in these strains. The specific activity of RBC catalase in C57BL, C57BL/6 and NBL is 2.5×10^7 M^{-1} sec^{-1} while that of BALB/c is $4.0 \times 10^7 \times 10^7$ M^{-1} sec^{-1}. The relative concentration of catalase is approximately 30% lower in NBL erythrocytes compared with the other 3 strains. Thus, the high RBC catalase activity of BALB/c is due to a high specific activity plus a high intracellular enzyme concentration; the medium RBC activity of C57BL and C57BL/6 is due to a low specific activity and a high intracellular enzyme concentration; and the low enzyme activity of NBL is due to a low specific activity and a low intracellular concentration.

Isozymes of kidney catalase, which were shown to be controlled by a single autosomal locus with two alleles, appear to be linked with the H-2 locus on chromosome 17. Experiments with H-2 recombinant strains (strains with crossovers within the H-2 complex) have shown that the locus for kidney catalase isozyme is located between Ss-Slp and Tla--which encompasses the H-2D end of the histocompatibility complex.

Linkage experiments to determine the exact location of kidney catalase isozyme on chromosome 17 have led to the development of a convenient and novel method for cytotoxic H-2 typing. Splenic lymphocytes, either from fresh or frozen tissue, are used to absorb the anti-H-2 antibody used in a cytotoxic test. If the absorbing spleen has the desired H-2 antigen, there will be no lysis in the test system; if the absorbing spleen does not have the desired H-2 antigen, then there will be complete lysis in the test system. Attached is an example of the cytotoxic absorption test for the H-2K haplotype using two inbred strains and their F_1 hybrid. Using frozen tissue in the cytotoxic absorption test enables one to perform the assay at their convenience and also allows the shipping of test material to another laboratory for H-2 typing.

Biochemical markers in inbred strains of mice used in cancer research. A joint program with J. Hilgers to construct a genetic profile of the inbred strains of mice in the United States and Europe used in cancer research is continuing.

In collaboration with E. A. Boyse, we established that a mutation of the G_{IX} antigen (the major glycoprotein component of the MuLV envelope) recently found in a C57BL/6 subline was a valid genetic change rather than a result of an outcross in the stock. Perfect agreement was found between the mutant C57BL/6 stock and C57BL/6Hf for 11 enzyme loci and 5 surface cell antigen loci.

Inheritance of the expression of MTV group specific (gs) antigens. In collaboration with S. Nandi, linkage analysis to locate gene(s) associated with MTV expression in milk and mammary gland tissue is being conducted. The GR high tumor incidence strain was crossed with C57BL, I, and BALB/c. At the present time first generation backcross females are being examined.

Data are being collected on the titer of MTV antigen in milk from C3H sublines, GR, 2 C57BL sublines, 2 BALB/c sublines, and I. First lactation milk from C57BL had zero titer and first lactation milk from GR had a titer of 1024. The Immunofluorescence Absorption test (IFA) is being used in this study.

Significance for Cancer Research: (NCP Objective 3, Approach 3)

This study provides an animal model for the determination of genetic and non-genetic factors for human breast cancer.

Proposed Course of Project: Three-point linkage analysis is to determine the exact position of kidney catalase isozyme on chromosome 17. The reference markers will be the private antigens of $H\text{-}2D^k$ and the TLA thymocyte antigen.

Experiments to characterize the biochemical events during mammary gland differentiation and tumorigenesis have been initiated. The experimental design to obtain information (in vivo) from high incidence tumor strains and low incidence tumor strains for time of appearance and concentration of specific enzymes and polyamines during mammary gland development, lactation, and tumorigenesis, and to correlate this with appearance and concentration of MTV antigen in milk and mammary tissue. This information then will be used to design experiments to study these events in mammary gland organ culture where we can control development and tumorigenesis by altering the chemical and hormonal environment.

Associated Activities: Assistant Project Officer for 5 contracts awarded by the Experimental Biology Committee of the Breast Tumor Task Force. The projects are in the areas of the role of hormones in tumorigenesis and the characterization of mammary gland epithelial cells.

Participation on the Board of Editors of the Journal of the National Cancer Institute as an Associate Editor.

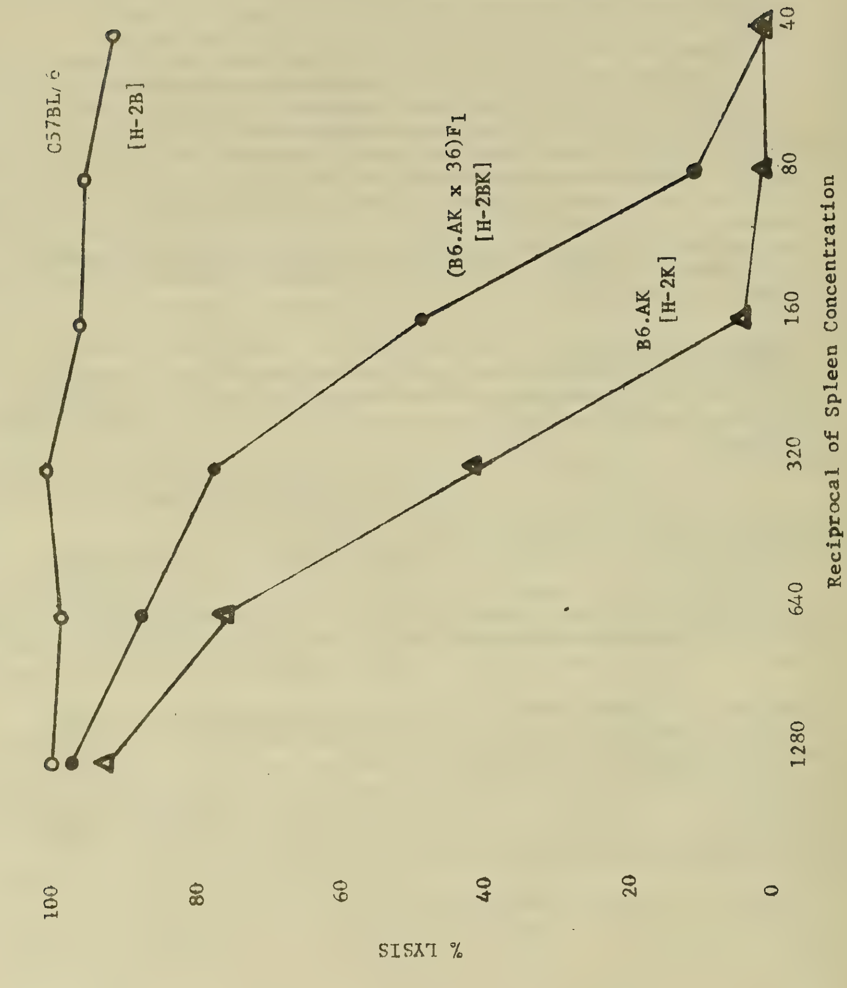
Publications:

Grieshaber, C. K. and Hoffman, H. A.: A rapid and convenient method for isolation of murine erythrocyte catalase by gel filtration and DEAE-cellulose chromatography. Anal. Biochem. 60: 537-544, 1974.

Hoffman, H. A. and Grieshaber, C. K.: Genetic studies of murine catalase: liver and erythrocyte catalase controlled by independent loci. J. Hered. 65: 273-275, 1974.

Grieshaber, C. K. and Hoffman, H. A.: Genetic regulation of the expression of catalase activity in murine red blood cells. Biochem. Genet. 14, in press, 1975.

Cytotoxic Absorption - H-2K Haplotype



Serial No. Z01 CBD 3251-04 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Effect of Diet and Bedding (Cedar Shavings) on Occurrence of Mammary Tumors and Hepatomas in Mice

Previous Serial Number: Same

Principal Investigator: W. E. Heston

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description: Objective 1. Approach 4

Objective: To determine the reason why mice from our high mammary tumor and high hepatoma strains of mice, C3H-A^{vy} and C3H-A^{vy}fB, failed to get these tumors when transferred to Dr. Sabine's laboratory in Australia, especially to determine if it was due as Dr. Sabine determined to cedar shavings that we used and he did not. He concluded that cedar shavings are carcinogenic.

Methods: The two obvious factors by which his colony care differed from ours 1) food, his Charlick's diet vs. our Purina Lab Chow, and 2) bedding, his pine sawdust vs. our pine sawdust plus cedar shavings, were tested to see if they had any influence on the incidence of mammary tumors or hepatomas in C3H-A^{vy} and C3H-A^{vy}fB mice in this laboratory.

Results: The occurrence of both hepatomas and mammary tumors was even slightly increased when the mice were fed his Charlick's diet compared with the Purina Lab Chow so the food obviously was not a factor in the great reduction of these tumors in his laboratory. The slight increase was due to the fact that the mice fed the Charlick's diet gained weight slightly faster probably because the Charlick's pellets were a little softer and the mice ate more of them.

There was no difference in occurrence of either mammary tumors or hepatomas when the mice of both strains were bedded with pine sawdust alone compared with pine sawdust plus cedar shavings when care was taken to prevent those on pine alone from becoming infested with ectoparasites and thus, there was no difference between the two groups in body weight. There was no evidence whatsoever that cedar shavings are carcinogenic. It is obvious that his animals did not get tumors because when bedded without the cedar they became so badly infested with ectoparasites that they did not grow well. Anything that reduces growth of animals reduces occurrence of tumors other factors being equal.

Significance to the Program of the Institute:

The demonstration that Sabine's conclusion that "cedar shavings were carcinogenic" was false is extremely important in view of the fact 1) that for over 25 years investigators at NCI have been using cedar shavings for their experimental animals in studies, many of which were carcinogenic studies, 2) that cedar shavings are used as bedding for pets throughout this country, and 3) human beings have been exposed for many years to cedar wood and cedar wood products especially cedar wood oil.

Proposed Course: The study has been concluded and written up and will soon appear in JNCI.

Publication:

Heston, W. E.: Brief Communication: Testing for possible effects of cedar wood shavings and diet on mammary gland tumors and hepatomas in C3H-A^{vy} and C3H-A^{vy}fB mice. J. Natl. Cancer Inst. 54, March, 1975

Addendum:

Key word descriptors: Mammary tumors
Hepatomas
Carcinogenesis
Cedar shavings
Diet

Identification No. Z01-CBD 03253-06 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Influence of Age and Sex on Carcinogenesis Induced by Chemicals in Buffalo Strain Rats

Previous Serial No.: Same

Principal Investigators: Kurt J. Stromberg and Melvin D. Reuber

Man Years:

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Key word descriptors: Influence of age and sex on susceptibility of rats to chemical carcinogenesis

Objective:

Reports have been made of studies on the influence of age and sex in hepatocarcinogenesis in Buffalo strain rats ingesting diethylnitrosamine (J. Nat. Cancer Inst. 41: 1133-1140, 1968), N-4 (4'-fluorobiphenyl) acetamide (J. Nat. Cancer Inst. 41: 1047-1054, 1970), and 3'-methyl-4-dimethylaminoazobenzene (J. Nat. Cancer Inst. 48: 675-683, 1972). Current studies have been directed toward the age and sex incidence, and histopathology, of breast tumors induced in Buffalo rats following ingestion of N-4(4'-fluorobiphenyl) acetamide. In addition, a study of the carcinogenicity of N-nitrosomethylurea on Buffalo rats of varying ages has been undertaken.

Methods Employed:

Inbred Buffalo strain male and female rats (12 to 16 rats per group) of ages 4, 8, 12, 24, and 52 weeks had ingested 0.04% N-4(4'-fluorobiphenyl) acetamide (4'-F-4BAA) in a semi-synthetic diet for 56 weeks. 12 weeks later the animals were killed and a complete autopsy was performed. In the case of N-nitrosomethylurea, similar numbers of Buffalo rats of the same ages ingested 0.0014% N-nitrosomethylurea for 3 months, the animals were then sacrificed, and a complete autopsy was performed.

Major Findings:

Among inbred Buffalo rats ingesting 4'-F-4BAA, susceptibility to mammary carcinogenesis was related to the age and sex of the animals. Young female rats developed most mammary tumors. Approximately one-half of these were well-differentiated adenocarcinomas while the remainder were either poorly differentiated or anaplastic adenocarcinomas.

Gross autopsy and light microscopic findings on rats ingesting N-nitrosomethylurea indicated this carcinogen, when administered at this concentration, possessed minimal hepatocarcinogenic potential in any of the age or sex groups. However, male animals in younger age groups infrequently had sarcomas of the stomach and intestinal tract.

Significance of the Program to the Institute:

National Cancer Plan: Objective 3, Approach 3

This series of pathologic studies is the first to evaluate the fundamental biologic parameters of age and sex in a uniform and comprehensive manner with representatives of three widely different classes of chemical carcinogens.

Publications:

Stromberg, K. and Reuber, M.D.: Histopathology of breast lesions induced in Buffalo strain rats of varying ages by ingestion of N-4(4'-fluorobiphenyl) acetamide. J. Nat. Cancer Inst. 54, May 1975.

Identification No. Z01 CBD 03254-06 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of a Representative Animal Type-C RNA Tumor Virus, Avian Myeloblastosis Virus (AMV), in Order to Develop Methods for Detection of Similar Viral Information in Human Tissues

Previous Serial No.: Same

Principal Investigator: Kurt J. Stromberg, M.D.

Man Years:

Total:	4
Professional:	2
Other:	2

Project Description:

Key word descriptors: Characterization of core and ribonucleoprotein components of an avian RNA tumor virus

Objective:

I. Isolation of the AMV core component. II. Identification and localization of enzymatic capacities in the AMV virion. III. RNA and polypeptide composition of the AMV component. IV. Fractionation of the AMV core component and characterization of its ribonucleoprotein (RNP) constituents. V. Determination of the biologic capacity of AMV core component. VI. Application of these methods to detection of type-C particle information in human cancer tissue.

Methods Employed:

The experimental design has been to fractionate AMV into its component parts by two successive methods: firstly, by exposure of intact AMV to a nonionic surfactant which separates the viral "envelope" from the viral "core", and secondly, by exposure of the resulting viral cores to high concentrations of sodium chloride which releases the inner viral RNP, or "nucleoid." The three viral components are separated by ultracentrifugation and then characterized by electron microscopic, biochemical, enzymatic methods, and biologic methods.

Major Findings:

Recent work has focused on a method for selective release and partial purification of the DNA polymerase activity of the core component of AMV by exposure to sodium chloride. A greater than 10-fold purification in this reverse transcriptase activity is rapidly achieved in this manner which, in addition, releases those several polypeptides which appear to comprise the inner ribonucleoprotein of the AMV core. The presence of a small, but consistent, amount of DNA has been identified in AMV cores. However, preparations of AMV cores are not leukemogenic when injected into the peritoneal cavities of neonatal chicks of a susceptible genetic strain.

Significance of this Project to the Institute:

National Cancer Plan: Objective 5, Approach 4

Early detection of preclinical cancer in man represents a major objective of current cancer research. Rapid, simple, and quantitative methods have been developed for separation of the envelope, core, and RNP components of a representative C-type RNA tumor virus. The possible association of type-C RNA tumor viruses in human cancer dictate that the structural, biochemical, and enzymatic characteristics of animal type-C viruses be determined as a prerequisite to detection of similar viral-like characteristics in human fluids and precancerous tissues. As an example, the unique and important advantage of salt treatment of AMV cores is that the DNA polymerase-containing core is separated from possible non-viral contaminants and membrane components before release of the DNA polymerase by sodium chloride. This procedure, therefore, may assist in the detection of viral particle-associated DNA polymerase activity in crude biological materials such as human tumor extracts, samples of human milk, etc. These procedures of fractionation and characterization can now be applied on human material to glean information on the possible association of type-C viruses to cancer in man.

The presence of small amounts of DNA in AMV core preparations was unexpected. Whether this represents trapping of extraneous host cell DNA during virus maturation, or is pertinent to viral replication can be evaluated by experiments which determine if this core-associated DNA hybridizes to viral RNA or host DNA.

The absence of leukemogenic activity in AMV core preparations suggests the possibility that cores may be used to immunize against the development of leukemia in susceptible chickens. Were an RNA tumor virus of indisputable human origin to be induced, and its oncogenicity were established in man, this biologic observation with chickens as an animal model would assume considerable importance.

Publications:

Stromberg, K. and Wilson, S. H.: Structural studies of avian myeloblastosis virus: Fractionation of the core component with selective release of polypeptides and partial purification of the associated DNA polymerase activity. *Biochim. Biophys. Acta* 361: 53-58, 1974.

Stromberg, K.: Structural studies of avian myeloblastosis virus: Absence of leukemogenic activity of core component. *Proc. Soc. Exptl. Biol. Med.* 146: 1111-1113, 1974.

Deeney, A. O'C., Stromberg, K., and Beaudreau, G. S.: Identification of DNA in the core component of avian myeloblastosis virus. *Biochim. Biophys. Acta*, in press.

Serial No. Z01 CBD 3256-04 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructure, Biology, Biochemistry, and Immunology of Mammary Tumor Virus Host-Cell Interaction

Previous Serial Number: Same

Principal Investigator: Gilbert H. Smith

Other Investigators: David G. Longfellow

Man Years:

Total:	3-1/4
Professional:	1-1/4
Other:	2

Project Description: The objective of the project is to elucidate the aspects of cell-virus interaction in mammary tumor virus (MTV) infection and tumorigenesis. The approach or methods involve the isolation and purification of intracytoplasmic A type particles (MTV nucleoprotein cores) from primary and transplanted tumors and the purification of B particles (mature MTV virions) from primary mouse mammary tumors. Biochemical, immunologic and biologic analysis and comparison of these two types of particles are pursued in an attempt to establish an ontologic relationship between them and to assess their relative role in mammary neoplasia in mice.

Major Findings: A high molecular DNA unique to intracytoplasmic A particle was found associated with the purified particles. A manuscript describing this work was published in Cancer Research.

Characterization of the A particle DNA by hydroxyapatite column chromatography, by base analysis, by labeling characteristics, by analysis in the Model E Beckman analytic ultracentrifuge and by electron microscopy has been completed. A manuscript describing these studies has been submitted for publication.

In addition to DNA, RNA was also found to be a component of purified intracytoplasmic A particles. In contrast to the DNA and to RNA found in MMTV(70S), it was of relatively low molecular weight. Enzymatic studies with the intact particles indicate that the RNA is not susceptible to degradation prior to extraction and further that some of it may be heteroduplexed with A particle DNA.

In earlier publications we reported our SDS-polyacrylamide analysis of A particle structural proteins and comparison with those of MMTV. Unexpectedly, there was considerable disparity in the molecular sizes of the polypeptides composing each particle. In particular, the major structural proteins of the viral nucleoid (p28 and p14) were missing in A particle gels. We have completed a study to determine whether this disparity was due to the presence of uncleaved MMTV structural proteins in the A particle. This was confirmed and a manuscript describing these studies has been submitted for publication.

Incorporation of ^{3}H-thymidine (a DNA tracer) into intracytoplasmic A particle containing transplantable testicular tumors was assessed by light and electron microscopic autoradiography. Statistical analysis of grain distribution in the autoradiographs strongly supported our biochemical and biophysical studies demonstrating the presence of DNA in A particle inclusions. The autoradiographic data was in turn supported by analysis of the specific activities (cpm/mg DNA) of A particle vs. nuclear DNA from the same tumors as did the buoyant density analysis of the respective DNA's on the analytical ultracentrifuge. The studies are complete; a manuscript is in progress.

Significance for Cancer Research: (NCP Objective 3. Approach 3)

The mouse mammary tumor virus problem has been and is being used as a relevant model for understanding human mammary neoplasia. Yet our knowledge of the intracellular events in MTV infection is essentially nil. The project is designed to provide a base to further our understanding of the intracellular events with the aim of elucidating the replicative cycle of MTV. Subsequently, it is hoped, armed with this information, to specify the tumorigenic influence of the virus.

Publications:

Smith, G. H., Litwack, M., and Longfellow, D. G.: High molecular weight DNA associated with intracytoplasmic A particle inclusions. Cancer Res. 34: 2654-2662, 1974.

Noseworthy, J., Smith, G. H., Himmelhoch, S. R., and Evans, W. H.: Protein and glycoprotein electrophoretic patterns of enriched fractions of primary and secondary granules from guinea pig polymorphonuclear leukocytes. J. Cell Biol. 1975, in press.

Gantt, Raymond, and Smith, Gilbert H.: RNA methylase activity in virus-infected normal and neoplastic mouse mammary tissue. In Borek, F., Salvatore, F., Schlenk, F., Williams-Ashman, G., and Zappia, V. (eds.): The Biochemistry of S-adenosyl-methionine. New York, Columbia University Press. 1974.

Keyword Descriptors: Mammary Virus, Intracytoplasmic A Particles, Mammary Neoplasia, Virus-Cell Interaction

Serial No. Z01 CBD 3260-04 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Part I. Genetic Transmission of the Mammary Tumor Virus in Mice

Previous Serial Number: Same

Principal Investigator: W. E. Heston

Other Investigators: Wade Parks

Man Years:

Total:	2-1/2
Professional:	1
Other:	1-1/2

Project Description: Objective 1. Approach 1

Objective: To ascertain whether or not the mouse mammary tumor virus information is transmitted as a single gene.

Methods: The principal part of this study is the breeding test of backcross segregants. Strain GR males with MMTV were mated to C57BL females without MMTV. The F_1 males were then mated to C57BL females to produce first backcross mice. Twenty-five first backcross males and 25 females have been backcrossed again to C57BL in an attempt to produce 25 second backcross females for each BC_1 male and as many from each BC_1 female as possible. Results from these BC_2 females would indicate the genotype of the BC_1 female. F_2 females were also produced. Milk taken from all females is being assayed for MMTV antigen by Dr. Wade Parks. Results of these assays will later be correlated with occurrence of mammary tumors.

Results: The occurrence and segregation of the antigen in the parent strains, the F_1, F_2, and BC_1 generations are in line with an interpretation of a single dominant gene controlling production of MMTV. This seems to be confirmed by the BC_2 females except for the occurrence of intermediate females with titers of antigen intermediate between that in GR and C57BL.

Significance of Program to the Institute:

It is very important to know how this virus is being transmitted aside from milk transmission. Is it transmitted genetically and if so, is it transmitted as a single gene? If the viral information is shown to be transmitted as a definite locus in the host genome this will be extremely important in guiding our search for a breast cancer virus in women.

Proposed Course of Study:

This project will require from 12 to 18 months yet for completion. Many more females' milks must yet be tested and then the females will have to be observed for later development of tumor. We must first determine whether we are getting clear-cut segregation of antigen or is it a quantitative variation. Second we must determine whether the results of segregation indicate a single dominant gene. Third we must see how well presence of antigen correlates with development of tumor.

Project Title: Part II. Studies on the Mouse Mammary Tumor Virus.

Principal Investigator: W. E. Heston

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description: Objective 1. Approach 4

Objective: The aims of this study are:

1) To ascertain if genetically resistant strain C57BL has MMTV.

2) To see if C57BL can propagate MTV from other strains.

3) Does MTV have any influence on chemical carcinogenesis?

4) Do chemically induced mammary tumors contain MTV?

Methods:

The following sublines of mice have been produced:

C57BLfRIII - The C57BL with RIII MTV to see if this MTV is propagated.

C57BLfC3H - The C57BL with C3H MTV to compare with C57BLfRIII.

BALB/cfC57BL - The BALB/c with C57BL MTV, if there is a C57BL MTV. This is the test for MTV in C57BL.

In addition, C3H with MTV and C3HfB without milk transmitted MTV have been injected with MCA to see if the presence of MTV in C3H alters the tumor induction capacity of MCA. Also, BALB/c without MTV and BALB/cfC3H with MTV have been injected with MCA.

C3HfB females without milk transmitted MTV were fed heptachlor and heptachlor epoxide to induce mammary tumors which would then be tested for titer of MTV.

Results: Some mice in each of these experiments except the last are still living and until they are autopsied final results cannot be tabulated.

The heptachlor and the heptachlor epoxide did not induce mammary tumors in the C3HfB mice thus failing to confirm that they are carcinogenic for the mammary gland at the low concentration used. All the females fed the heptachlor epoxide, however, and living to advanced age did have hepatomas confirming that even at these low concentrations this compound is carcinogenic in the liver.

Significance of Program to Institute:

These projects are a part of the overall study of the nature of the mouse mammary tumor virus and its relationship to the genome of the host and to introduced chemical carcinogens.

Proposed Course of Study:

During the coming year these studies should be concluded.

Publications:

Heston, W. E.: Genetics of Cancer. The Wilhelmine E. Key 1974 Invitational Lecture. J. Hered. 65: 262-272, 1974.

Heston, W. E.: Genetic Aspects of Human Cancer. In Klein, G. and Weinhouse, S. (eds.): Advances in Cancer Research. New York, Academic Press, Inc. In press 1975.

Addendum:

Key word descriptors: Mouse mammary tumor virus
Genetic transmission of MMTV
MMTV antigen
Mammary tumors

Identification No. Z01 CBD 03267-03 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of DNA Methylation Activity in Subnuclear Fractions Prepared from Mouse Cells in S Phase

Previous Serial Number: Same

Principal Investigator: David G. Longfellow, Ph.D.

Other Investigators: R. Raymond Gantt, Ph.D.
Gilbert H. Smith, Ph.D.

Cooperating Unit: Cellular Physiology and Oncogenesis Section
Laboratory of Biochemistry
National Cancer Institute

Man Years:

Total:	1
Professional:	3/4
Others:	1/4

Project Description:

Key Word Descriptors: DNA methylation, Nucleic acid methylation, Cell control mechanisms, Cell cycle, and S phase

Objective: To determine the relationship of DNA methylation to DNA replication and neoplasia.

Methods: An established mouse embryo fibroblast cell line is carried in culture as two sublines, one "normal," the other neoplastic. Neoplastic conversion is induced by supplementing the culture fluid with horse serum rather than fetal calf serum.

Methods: (Continued)

In current studies, utilizing the normal subline, cells are synchronized to the S phase of the cell cycle with Amethopterin. Prepared nuclei are disrupted by nitrogen cavitation and fractionated by discontinuous gradient techniques. The presence of DNA methylase is detected by the ability of a subfraction to transfer a labeled methyl group from S-adenosylmethionine to an endogenous or exogenous DNA acceptor. The acid washed DNA reaction products are hydrolyzed in formic acid and the base products are analyzed by high performance liquid chromatography.

Major Findings: 50% of the nucleic acid methylating activity was found in the nuclear subfraction rich in membrane-bound DNA together with 70% of the newly synthesized DNA. The major particulate DNA polymerase activity was demonstrated in this nuclear fraction also. Methylation of DNA bases represented approximately 1/2 of the nucleic acid methylation activity when M. lysodeikticus DNA was the exogenous acceptor. During S-phase of the cell cycle 5-methylcytosine was found as the major modified base in the DNA polymer which confirms observations by other investigators. In addition, another base, which chromatographs as a methylated form of guanine, was also observed in significant amounts during S phase. Preliminary work with similar fractions prepared from cells in G_1 revealed two greatly increased methylation peaks which had always been minor in S phase preparations and 5-methylcytosine activity was found reduced to 1/20 of that in S phase suggesting cell cycle specific fluctuations in nucleic acid methylation. A manuscript describing these studies has been submitted for publication.

Significance for Cancer Research: NCP Objective 3. Approach 3

The development of this particulate, in vitro system for studying the nucleic acid methylation capacity of cells should enable us to compare with a high resolution system the methylation patterns between different cell types, and between stages of the cell cycle. A comparison study of DNA methylation by the paired normal and neoplastic cell lines developed in this laboratory should provide a better understanding of the effects of neoplasia on the process of DNA methylation.

Awards: NCI Postdoctoral Research Fellowship to David G. Longfellow, Ph.D.; 1 F22 CA 01072-01

Publications:

Smith, G. H., Litwack, M., and Longfellow, D. G.: High molecular weight DNA associated with intracytoplasmic A particle inclusions. Cancer Res. 34: 2654-2662, 1974

Longfellow, D. G., and Steinberg, R. L.: A turntable device for the determination of radial symmetry by photographic rotation. Trans. Amer. Micros. Soc. 94: 124-128, 1975

Serial No. Z01 CBD 3268-03 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation of Enzyme Expression in Normal Differentiating and Neoplastic Tissue

Previous Serial No.: Same

Principal Investigator: Charles K. Grieshaber

Other Investigators: Harold A. Hoffman
Warren H. Evans (Laboratory of Biochemistry, NCI)
M. R. Banerjee (Tumor Biology Laboratory, Univ. of Nebraska)
E. A. Boyse (Sloan Kettering Institute)
J. Hilgers (Netherlands Cancer Institute)
S. Nandi (Cancer Research Laboratory, Univ. of California, Berkeley)

Cooperating Units:

Laboratory of Biochemistry, NCI
Transplantation Immunology Branch, NIAID (Supplied allotypic H-2 typing sera)
Office of Program Resources and Logistics, DCCP, NCI (Supplied rabbit anti-MTV serum)

Man Years:

Total: 3
Professional: 1-1/2
Others: 1-1/2

Project Description:

Objectives: To study the appearance or loss of enzyme activities and their substrates and products in normal tissues and tumors, thus, defining some of the chemical reactions involved in normal cellular differentiation and subsequent deviations during tumorigenesis. Similarities and differences between those processes (both intra- and extra-cellular) which regulate protein levels and enzyme activities in normal differentiated, growing, and neoplastic cells is essential to our understanding the biology and chemistry of cancer.

Methods: Mammary gland and tumors, liver and hepatomas, kidney, and bone marrow and peripheral blood cells are used as the source of proteins upon which appropriate physical, biochemical, genetic, and immunochemical analyses are carried out. Enzymes currently under investigation are catalase, peroxidase, arginase, ornithine decarboxylase, S-adenosyl-L-methionine decarboxylase and adenyl cyclase--those enzymes whose modulations are influenced by various regulatory factors. Mice from eight inbred strains and strain 13 guinea pigs are commonly used as the source of tissues.

Major Findings:

1) In an effort to describe the mechanism responsible for interstrain differences in electrophoretic mobilities of kidney catalase, a strong correlation was found between electrophoretic migration and histocompatibility (H-2). Two electrophoretic forms of the enzyme, aggregated (type b) and nonaggregated (type a), are correlated with histocompatibility type. The type a catalase is correlated with H-2K, the type b catalase is associated with the other histocompatibility types. This genetic association was unexpected but may lead to some understanding of the relationship between the H-2K tissue type and MTV susceptibility and elevated levels of catalase enzyme in mammary tumors.

2) Studies on arginase levels in strain 13 Guinea Pig bone marrow granulocytes were carried out in an effort to determine the significance of the increase in enzyme activity during cell differentiation. These studies led to investigations of ornithine metabolism in immature and mature bone marrow granulocytes. It was discovered that arginase increases about 5-fold as a cell matures, whereas the ornithine metabolizing enzymes: Ornithine amino transferase and Ornithine decarboxylase decrease 3- and 13-fold respectively during development. The polyamine ratios (Spermidine/Spermine) of the mature and immature cell populations vary significantly. The immature cells contain two times as much spermidine as do the mature cells. There is no difference in spermine content in the two cell types. Since ornithine decarboxylase converts ornithine to putrescine, it is the first enzymatic step in the biosynthesis of the polyamines. The asynchronous appearance of arginase, which forms ornithine, and ornithine decarboxylase, which converts ornithine to polyamines is not yet understood. However, experiments are underway which should ascertain the role of ornithine and the polyamines in regulation of bone marrow granulocyte differentiation.

3) Preliminary studies on the polyamine content of mammary glands during pregnancy and lactation and in mammary tumors show that the spermidine concentration is 12-15 times greater in C3H-A^{vy} tumors (240-290 nmole/gm) compared to CR tumors (20 nmole/gm) compared to the GR

tumor (25-28 nmole/gm). During pregnancy and lactation the polyamine content of mammary glands from both strains increase to a level approximately 4-5-fold above resting gland levels. The increase in polyamines during tumor formation is currently being studied with respect to Mammary Tumor Virus (MTV) infection as measured by the appearance of high titers of MTV antigen on immunofluorescence assay.

4) The specific activities of red blood cell catalase from four inbred strains of mice (BALB/c, C57BL, C57BL/6 and NBL) were measured on purified preparations. The specific activity in the NBL strain and the C57BL sublines was $2.5 \times 10^7 M^{-1} sec^{-1}$; that of BALB/c erythrocyte catalase was $4.0 \times 10^7 M^{-1} sec^{-1}$. The intracellular concentration of catalase (measured immunochemically) is equal in the BALB/c, C57BL and C57BL/6 strains, whereas the concentration is 30% lower in RBC's from the NBL strain. The high overall catalase activity of BALB/c RBC catalase is due to a high specific activity plus a high intracellular concentration. The intermediate catalase activity found in RBC's from the C57BL sublines is due to a low specific activity plus a high concentration. The low catalase activity of NBL erythrocytes is the result of both a low specific activity and low concentration of the enzyme.

5) Loci controlling liver catalase activity (Cs-1) were shown to be genetically independent. The Cs-1 locus, located on chromosome 2, is approximately 26 centi morgans from the agouti locus. This locus was determined to control the wide variation in erythrocyte catalase levels found in various strains of inbred mice.

6) Studies on differential gene action with respect to catalase and myeloperoxidase in bone marrow WBC's and peripheral RBC's showed that the regulatory effect of the Cs-1 locus takes effect after the marrow stem cell is committed to the erythroid line of development. There also appears to be a compensation in H_2O_2 metabolizing enzymes where in the case of low or absent RBC catalase, the WBC's from the same animal have an elevated level of myeloperoxidase.

Significance for Cancer Research: NCP Objective 3. Approach 3

This project provides a model system in tissues from animals of known genetic composition for the study of protein and enzyme regulation and interaction during normal differentiation and neoplastic transformation. This system may have wide significance to general processes of differentiation and tumorigenesis since understanding the several potential points in the regulation of key enzymes will provide a description of metabolic sequences and biochemical lesions.

Proposed Course:

1) Polyamine concentration and rates of synthesis will be determined in mammary tissue with regard to:

a) The appearance of MTV antigens in the tissue.

b) Hormone dependency or independency of mammary tumors.

c) The effect of inhibitors of polyamine synthesis on tumor progression.

2) The role of polyamines as regulators of normal and leukemic bone marrow cell differentiation will be investigated in more detail with emphasis on their role in maintaining cells in the non-differentiated state.

3) Biochemical and genetic studies will be continued to determine the connection between H-2K histocompatibility and catalase aggregation. The reason for aggregation will be studied.

4) Bone marrow cells from normal guinea pigs will be cultured along with those from leukemic mice to study the involvement of cyclic nucleotides (cAMP and cGMP) in normal and abnormal differentiation.

Publications:

Grieshaber, C. K. and Hoffman, H. A.: A rapid and convenient method for isolation of murine erythrocyte catalase by gel filtration and DEAE-cellulose chromatography. Anal. Biochem. 60: 537-544, 1974.

Hoffman, H. A. and Grieshaber, C. K.: Genetic studies of murine catalase liver and erythrocyte catalase controlled by independent loci. J. Hered. 65: 273-275, 1974.

Grieshaber, C. K. and Hoffman, H. A.: Genetic regulation of the expression of catalase activity in murine red blood cells. Biochem. Genet. 14, in press, 1975.

Key Word Descriptors: Mammary Tumor Virus, Inbred Mice, Bone Marrow Granulocytes, Amino Acids, Leukopoiesis, Polyamines, Erythrocyte Catalase, Regulatory Enzymes, Histocompatibility, Biochemical Genetics

Serial No. Z01 CBD 3270-02 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Relationship of Genetic and Non-genetic Factors in Mammary Tumorigenesis in Mice

Previous Serial Number: Same

Principal Investigator: G. Vlahakis

Other Investigators: W. E. Heston and G. H. Smith

Man Years:

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description: Objective 1, Approach 4

Objectives: To study the relationship between genetic factors and the milk-borne viral influence (MTV) in the occurrence of mammary tumors.

Methods and Results: The testing of low oncogenic DD-MTV from mammary tumor strain DD, in strain C3H-A^{vy}fB is almost complete. The viable yellow gene (A^{vy}) which characterizes C3H-A^{vy}fB is an example of the role of genes in tumorigenesis. The data will be tabulated and analyzed for publication.

Methods and Results: The testing of the mammary tumor virus from strain C3H in strain C57BL/M is now complete. Although C57BL/M has been shown to be slightly more susceptible to the development of both spontaneous and induced mammary tumors than the C57BL/He substrain, there was no increase in susceptibility to the virulent C3H-MTV.

Three lines of C57BL/MfC3H were started, fostered for only one generation on three different C3H females, all of which developed mammary tumors. From a total of 122 C57BL/MfC3H females in the three lines, there was only 1 tumor occurring at 13 months of age. It was a typical mammary tumor and it occurred in the first generation following the foster nursing. Electron microscopic examination revealed B particles characteristic of MTV, and the histologic diagnosis was adenocarcinoma type

B. The average age at death for all the females without mammary tumors was approximately 18 months.

Our results for C57BL/M are similar to the results reported in 1939 by Andervont for his subline of C57BL/An, from which our C57BL/He was derived in 1945. Andervont's experimental females of C57BL/AnfC3H consisted only of animals in the first generation following the foster nursing, and he observed just two mammary tumors. Thus, strain C57BL/M is also resistant to the oncogenic action of the virulent C3H-MTV, and this resistance is genetic in nature, as has been interpreted for C57BL/He when it was outcrossed to C3H. The C3H-MTV is not propagated and is lost after only a few generations following its introduction by strain C3H females which carry the milk-borne virus.

Methods and Results: We now have some results on the foster-nursed strain C57BL/MfDD (with DD-MTV). Moore and his co-workers at the Institute for Medical Research, Camden, New Jersey, have reported on the high incidence of mammary tumors occurring in strain C57BL/M with MTV from strain RIII. Since both strains RIII and DD are of European origin, and both develop hormone dependent mammary gland lesions, and therefore they may be related, we thought it worthwhile to test DD-MTV in C57BL/M.

Three females have developed mammary tumors at an average tumor age of 9 months. In one of the females there was also a hormone dependent mammary gland lesion which appeared to be regressing. She also had another mammary tumor. One of the mammary tumors examined by the electron microscope revealed B particles. In addition to the 3 females with mammary tumors there was another female that was found to have a hormone-dependent mammary gland lesion. Thus, to the list of strains that are capable of forming mammary gland lesions associated with pregnancy, the fostered strain C57BL/MfDD can be included. It has been some time since another mammary tumor has occurred. Like the results with C3H-MTV in C57BL/M, the DD-MTV may have been eliminated from C57BL/MfDD. Many of the breeders have been retired and are now being examined regularly for the occurrence of mammary tumors. We should have more conclusive information later this year on the fate of DD-MTV in strain C57BL/M.

Methods and Results: The fostered strain BALB/cfGR (with GR-MTV) has now been developed, and the objective here is to compare GR-MTV from the high European mammary tumor strain GR, with C3H-, A-, and DD-MTV's. BALB/c females are susceptible to mammary tumors when given MTV through foster nursing on strains that have the milk-borne virus. Enough Balb/cfGR females have now been set aside and they will be maintained as virgins to make possible a comparison with the earlier study, in which it was shown that the three naturally occurring MTV's from C3H, A, and DD ranged from high, to medium, to low ability in the production of mammary tumors only when compared in virgin BALB/c females. To date two mammary tumors have occurred in breeders at 10 and 11 months of age. There will be more information later this year on the occurrence of mammary tumors in the BALB/cfGR virgin females, and on how GR-MTV compares with the other lines of MTV.

Methods: Although we were not able to repeat the work of Hageman and Calafat of the Netherlands Cancer Institute, using their methods, in which they believe that they demonstrated strain BALB/c has a mammary tumor virus (MTV), we are again attempting it with a slightly modified experiment. Whereas the first time we maintained the females as virgins, this time we are breeding and allowing them to have as many litters as possible, since in general, breeding increases the occurrence of mammary tumors in female mice. This experiment, which consists of making cell-free homogenates or preparations from old mammary glands of retired breeders, and injecting the material into weanling animals, is now well underway and thus far not a single mammary tumor has occurred in the 25 BALB/c females after approximately 8 months following the injection. By the end of this year we should have enough data to answer the question of whether or not our subline of BALB/c carries an "overlooked" MTV as has been so described by Bentvelzen for apparently all sublines of BALB/c.

Methods and Results: Since the publication of Sabine, Horton, and Wicks in 1973, at the University of Adelaide, in Adelaide, Australia, in which they attributed the high spontaneous mammary and liver cancer in our strains C3H-A^{vy} and C3H-A^{vy}fB in the United States to the use of cedar shavings in the bedding material, we have been maintaining some C3H-A^{vy}fB females on pine alone. It is clear from early results that the mammary tumor incidence will be high in C3H-A^{vy}fB females on pine, and will probably be similar to the incidence for those females that we normally maintain on the mixture of both pine and cedar. Nine females on pine bedding have been necropsied with mammary tumors, the tumors occurring at an average age of 14.7 months, and in addition all had hepatomas characteristic of C3H-A^{vy}fB animals. In this study there will be approximately 50 C3H-A^{vy}fB females maintained on pine, and they will be particularly observed for the occurrence of both mammary and liver tumors.

Methods: It has recently been claimed by Tsubura et al., of Nara Medical College, Nara, Japan, that like strain GR in which MTV is transmitted by either sex, both female and male transmission of MTV occurs in their high mammary tumor substrain DD. In 1959 we started our line of DD, having obtained some breeding animals from Tsubura who had been a guest worker in our laboratory. In 1966 we made reciprocal crosses between strains DD and C57BL and found that mammary tumors occurred only in the (female DD x male C57BL)F_1 females, and not in the reciprocal (female C57BL x male DD)F_1 females. This was evidence that male transmission of MTV in strain DD does not occur. Since strain BALB/c was used in the production of the reciprocal hybrids by Tsubura and his group, we are repeating their work using our strain BALB/c. The experiment is underway and we are now saving the F_1 females of both types. We should have some indication later this year of whether or not male transmission of MTV occurs in DD when it is outcrossed to the susceptible BALB/c strain.

Methods: In 1971 we published a paper on the spontaneous cholangiomas occurring in our high hepatoma strain C3H-A^{vy}fB and in their hybrids. Later (1973) Turusov et al., who believe the tumors should be referred to as hepatoblastomas, and not cholangiomas, found an increase in their occurrence among mice receiving the pesticide DDT in the diet. We are now feeding DDT to some of our C3H-A^{vy}fB, both males and females, to see whether or not it will increase the incidence of cholangiomas. These interesting liver tumors have always been observed in association with hepatomas, and since strain C3H-A^{vy}fB is highly susceptible to the development of hepatomas in both sexes, it seemed worthwhile to test the effect of DDT in this strain. A group of control animals has also been included in this study feeding them our normal standard food. This experiment will be nearly complete by the end of this year and at that time we should have some indication of the effect of DDT in relation to the occurrence of cholangiomas in C3H-A^{vy}fB.

Again this year, collaborative studies on mammary tumors and hepatomas with other investigators both here and outside the NIH, are continuing. Our contribution of valuable mammary tumor and hepatoma mouse strains, and our knowledge of the biology of these neoplasms make such studies desirable, and therefore are actively being pursued. One in particular, with Dr. Rob Michalides of Meloy Laboratories, on molecular hybridization studies utilizing mouse MTV, is now complete, and we soon hope to have a manuscript ready to submit for publication. Also some time has been spent with other research workers both here and outside of the NIH, helping them to develop their own programs. This has consisted of discussions and of making available to them specific biological material for their own research requirements. Time has also been spent talking with students and their teachers who visit the laboratories at NIH as part of their learning experience.

Significance of Program to the Institute:

The occurrence of mammary tumors in mice depend on the interplay of many factors: the genotype of the animal, hormones, the mammary tumor virus (MTV), environment, etc. Studies of mammary tumors in mice need to be done so that eventually mechanisms of interaction between the various factors will be understood and therefore, quite possibly have relevance for the disease process in man.

Proposed Course of Experiment:

The study of the effect of low oncogenic DD-MTV from strain DD in the presence of the A^{vy} gene which characterizes C3H-A^{vy}fB will soon be complete, and the data will be tabulated and analyzed for publication.

The results of the fostered strain C57BL/MfC3H (with C3H-MTV) will be submitted as part of a future publication.

Key Words: Inbred mice, mammary tumor virus, genes, hormones

Identification No. Z01 CBD 03271-01 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Induction of Oncornavirus Expression in Mammalian Trophoblast Cells

Previous Serial No.: None

Principal Investigators: Kurt Stromberg, M.D. and K. V. Speeg, M.D., Ph.D.

Man Years:

Total:	4-1/2
Professional:	1-1/2
Other:	3

Project Description:

Key Word Descriptors: Oncornavirus induction, cell fusion, mammalian cells

Objective:

I. Induction of murine oncornavirus expression by cell fusion and chromosome segregation.

II. Induction of murine oncornavirus expression in trophoblast cells of a repressed mouse strain.

III. Induction by cell fusion and chromosome segregation of oncornavirus expression in human trophoblast cells.

Methods Employed:

The animal model system on which this project is based utilized explants of C57BL/6 mouse embryo brain that had been fused to an SV40 transformed human fibroblast cells. Oncornaviral expression was induced, presumably because of murine chromosome segregation. The electron microscopic evidence that trophoblast tissue of primate (including human) origin contained budding C-type oncornaviral particles has encouraged us to

Methods Employed (Continued)

apply the proven technique of viral induction by cell fusion to primate trophoblast cells grown in short term culture. Oncornavirus presence in culture fluid medium is detected by electron microscopy and assay for reverse transcriptase activity. An essential, and companion, assay for the presence of gene expression in trophoblast cells is radioimmune precipitation assay for human chorionic gonadotropin hormone, and viral p30 polypeptide.

Major Findings:

The capacity of cell fusion and resulting chromosome segregation to induce oncornavirus expression in hybrid clones of the repressed C57BL6 strain of mice has been established. Methods of culture of mouse trophoblast cells have been developed, and are being adapted to human trophoblast tissue.

Significance for Cancer Research:

National Cancer Plan: Objective 3; Approach 2

The relevance of the oncornaviral animal model to human cancer will be in question until a bonafide oncornavirus of human origin is growing in cell culture in adequate yield. Only then can appropriate detection techniques, such as radioimmune precipitation assays for viral polypeptides, be developed to correlate oncornaviral expression with neoplastic growth in man. Our project is devoted to this aim, namely, to gain human oncornaviral expression in cell culture.

Publications:

Minna, J., Gazdar, A., Iverson, G. M., Marshall, T., Stromberg, K., and Wilson, S.: Oncornavirus expression in human x mouse hybrid cells segregating mouse chromosomes. Proc. Natl. Acad. Sci. (USA) 71: 1695-1700, 1974.

Identification No. Z01 CBD 03272-01 LBGY
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control Mechanisms of Differentiated Functions in Normal and Neoplastic Trophoblast Cells

Previous Serial No.: None

Principal Investigators: K. V. Speeg, Jr., M.D., Ph.D.
Kurt Stromberg, M.D.

Cooperating Units: None

Man Years:

Total:	3
Professional:	2
Other:	1

Project Description:

Key Word Descriptors: Human choriocarcinoma, tissue culture, placental alkaline phosphatase, cyclic AMP, chorionic gonadotropin hormone

Objectives: 1) To maintain normal human trophoblast and human choriocarcinoma cells in tissue culture and to study their growth characteristics and changes in differentiated function under conditions of different media.

2) To study in detail modulation of two differentiated functions -- human chorionic gonadotropin hormone (HCG) and placental alkaline phosphatase.

Findings: Two lines of choriocarcinoma are being maintained in tissue culture and differences in growth characteristics are being found with different media. These differences include changes in expression of differentiated function. The effect of various modulation factors including cyclic nucleotides, steroid and peptide hormones, antimetabolites and inhibitors have been looked at in regard to alkaline phosphatase and HCG. Normal trophoblast tissue has been successfully grown in short-term explant culture.

Significance:

Objective 1 Approach 4
Objective 3 Approach 2
Objective 4 Approach 1
Objective 5 Approach 4

Knowledge of the basic biology of a particular kind of human cancer is a necessary prerequisite to understanding its etiology and control. In vitro growth of the normal differentiated cell and its neoplastic counterpart provides obvious advantages in comparing control mechanisms of the respective basic cell functions. In addition, changes in these basic functions under the influence of modulating factors such as oncornaviruses allows some statement to be made about the nature and origin of the neoplastic event. The control mechanisms of the two differentiated functions chosen for study (placental alkaline phosphatase and HCG) are poorly understood. They have the added advantage of potential use in early detection of certain human cancers of undifferentiated type.

SUMMARY STATEMENT
LABORATORY OF MOLECULAR BIOLOGY
DCBD, NCI
July 1, 1974 - June 30, 1975

Research in the Laboratory of Molecular Biology has been expanded by the addition of Dr. Seymour Wollman's Cell Organization Section and by the arrival of Dr. M. Rosenberg who has set up facilities to do RNA and DNA sequence analysis.

Currently the research effort is divided into four areas of interest:

1. What is the role of cyclic AMP in malignant transformation?

2. How is gene activity controlled and what are the nucleotide sequences at regions where transcription begins and terminates?

3. What is the mechanism of genetic recombination?

4. How does the thyroid cell form, store and release its hormone and what factors control its growth, differentiation and conversion to a malignant cell?

Cyclic AMP and Malignant Transformation--Over the last few years we showed that transformed embryonic fibroblasts had low levels of cyclic AMP and presented evidence that the decreased adhesiveness, rounded shape, rapid growth and increased agglutinability of transformed cells were due to their low levels of cyclic AMP. To understand why cyclic AMP levels are low in transformed cells, we have begun to characterize in detail the enzyme that synthesizes cyclic AMP, adenylate cyclase and the enzymes of cyclic AMP degradation, the cyclic AMP phosphodiesterases. In transformed NRK cells and CEF the activity of adenylate cyclase is diminished and this low activity appears to account for low cAMP levels. In some cases cAMP phosphodiesterase activity is also low. Decreased cAMP phosphodiesterase is secondary to low cAMP levels; cAMP is an inducer of cAMP phosphodiesterase. W. Anderson has identified one or more soluble intracellular proteins (MAC factors) that are able to activate adenylate cyclase. These factors may have a role in activating adenylate cyclase as the growth of NRK cells slows. Anderson has also been examining the changes in adenylate cyclase in transformation and finds that some viruses change the enzyme so that it no longer responds to one of the MAC factors.

Because of recent interest in cyclic GMP, we have begun to measure the levels of guanylate cyclase and cyclic GMP in cells. J. Nesbitt has found that guanylate cyclase is diminished in many types of transformation and is undetectable in Kirsten transformed NRK cells. Miller finds KNRK cells also have extremely low levels of cyclic GMP. These findings that transformation decreases cyclic GMP levels contradicts the widely held idea that cyclic GMP functions as a growth promoter.

M. Willingham has pursued ultrastructural studies on the effects of Bt_2cAMP on cell shape. He finds that cAMP induces the formation of thick bundles of microfilaments particularly at cellular margins and these are areas where the cell is closely opposed to the substratum. Using dark-field microscopy, Willingham also noted that transformed cells have microvilli which rapidly disappear upon addition of Bt_2cAMP. He has shown that the reason transformed cells are agglutinated by plant lectins is because microvilli interdigitate and hold cells together. cAMP treatment lowers agglutinability by causing resorption of microvilli.

Since transformed 3T3 cells appear to require diminished amounts of growth factors, P. Thomopoulos (NIAMDD) has been examining their capacity to bind the anabolic hormone insulin. He finds transformed cells bind considerably less insulin than untransformed 3T3 cells.

G. Johnson has begun to examine the energy metabolism of 3T3 cells by replacing glucose with other sugars. In general glucose is the only sugar that will support growth although a variant which will grow on galactose has been obtained.

K. Yamada has continued studies started elsewhere on the major cell surface protein (CSP) of chick embryo cells. He has improved the method of preparing CSP and finds CSP will function as an agglutinin. This suggests that CSP may function in cell:cell recognition.

Control of Gene Activity and the Structure of Regulatory Loci-- In order to understand how the cAMP-CRP complex enables RNA polymerase to initiate gene transcription S. Nakanishi has measured the effect of denaturing conditions, high temperature, low Mg^{++} or low salt, on the formation of a DNA:RNA polymerase preinitiation complex. Nakanishi's evidence indicates that cAMP-CRP acts by locally denaturing DNA at promoter loci. Another nucleotide that stimulates transcription is ppGpp (guanosine tetraphosphate). M. Aboud has found and purified a protein which is required for ppGpp to act.

Another approach to understanding how the cAMP-CRP complex acts is to determine the structure of CRP and the region of DNA to which it binds. We have developed a method of making large amounts of CRP and D. Schlesinger at Harvard Medical School is determining its sequence. Musso and de Crombrugghe together with Sklar and Weissman at Yale have been working at the sequence of the gal regulatory region. They have already succeeded in determining the sequence of the 5'-terminus of the gal mRNA from which they can deduce the sequence of the template DNA. They find there are 26 bases before the codon for the 1st amino acid of the 1st structural gene gal epimerase. This untranslated region has a high degree of twofold symmetry perhaps reflecting a regulatory function for the primer DNA.

S. Adhya and others have found that the product of λ gene (N) causes RNA polymerase to fail to obey normal transcriptional stop signals resulting in a very large and apparently stable species of RNA. It appears as if the N gene product antagonizes the action of termination factor *rho*. M. Rosenberg has been determining the nucleic acid structure involved in the control of transcriptional termination and developing new methods for sequence analysis. He has examined the 3'-terminal sequences of a number of transcripts from λ and λ*gal* templates and finds that a) all of the transcripts examined have 3'-terminal sequence heterogeneity consisting in the addition of 1-5 adenylate residues; b) termination factor *rho* results in a structural change in the termination sequence.

The Mechanism of Genetic Recombination-- M. Shulman has succeeded in isolating λ with mutant attachment sites. These mutations are in the region of homology between λ and *E. coli*. S. Gottesman has been investigating the functions required for λ integration and established that translation, transcription and replication are not required for the integration reaction. She has also developed a cell-free system in which λ prophage excision occurs. This system offers the possibility of identifying all the components required for the excision reaction.

Thyroid Cell Function--S. Wollman and co-workers have established that: a) the location of newly formed iodoprotein (30 sec after ^{125}I injection) is in the thyroid lumen indicating iodination is an extracellular event; b) ^{45}Ca^{++} is first taken up by the thyroid cell and later concentrated in the lumen where it binds to thyroglobulin and changes its physical properties; c) the thyroid epithelial cell can be induced to phagocytize red cells and debris from the thyroid lumen by a process different from macropinocytosis and apparently independent of TSH; d) mitosis of the thyroid cell can occur without the cell losing contact with adjacent cells in the follicle or the lumina or basal lamina. Also in collaboration with Dr. Hilfer at Temple University he has continued to study the origin of various cell types of the thyroid during embryological development.

Serial No. Z01 CBD 8000-05 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation of Gene Activity in E. Coli by Cyclic AMP

Previous Serial Number: Same

Principal Investigator: Ira H. Pastan

Other Investigators: Max Gottesman and Sankar Adhya

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	0.5
Professional:	0.5
Others:	0

Project Description:

Objectives: Cyclic AMP is required for the synthesis of a variety of enzymes in E. coli, most of which are inducible. Cyclic AMP acts at a transcriptional level. Our objective is to determine how cyclic AMP, cyclic AMP receptor protein (CRP) and other factors control gene activity.

Methods Employed: Studies on mRNA and enzyme synthesis have been carried out in intact cells and in cell-free extracts. Mutants which are defective in cyclic AMP metabolism have been isolated and characterized. Proteins controlling gene transcription are isolated and their mechanism of action studied.

Major Findings: In order to understand in detail how the cyclic-CRP complex promotes gene transcription, the structures of CRP and the region of DNA to which it binds need to be determined. With David Schlesinger at Harvard Medical School, we are trying to obtain the complete sequence of CRP. About 70% of the structure is now complete.

S. Nakanishi has investigated the structure of the promoters of the lac and gal operons by determining the effect of Mg^{++} concentration and temperature on the ability of RNA polymerase to initiate transcription at lac and gal promoters. He found that the requirements for cyclic AMP and CRP could be eliminated by allowing RNA polymerase to bind to the lac and gal promoters in the absence of Mg^{++}. Further some mutant promoters which were inactive in vivo could be activated by removing Mg^{++}. These findings suggest that the cAMP-CRP complex acts by destabilizing or melting promoter loci.

M. Aboud has investigated how the unusual nucleotide ppGpp acts to stimulate lac transcription. He has found that for ppGpp to stimulate transcription, a novel protein named TFms is required. Previously he showed that tRNA was also required. Thus maximal lac transcription requires cAMP, CRP, ppGpp, TFms and tRNA.

Significance to Biomedical Research and the Program of the Institute: National Cancer Plan Objective 2, Approach 3. As we understand in detail how the expression of bacterial genes is controlled we will be able to understand and then control the expression of the genes of animal cells and modify the behavior of cancer cells.

Proposed Course: To continue to identify nucleotides and regulatory proteins that control transcription.

Honors and Awards: None
Key Words: Cyclic AMP, gene expression, E. coli
Publications:

Musso, R., de Crombrugghe, B., Pastan, I., Sklar, J., Yot, P., and Weissman, S.: The 5'-Terminal Nucleotide Sequence of Galactose Messenger Ribonucleic Acid of Escherichia Coli. Proc. Nat. Acad. Sci. USA 71: 4940-4944, 1974.

Aboud, M., and Pastan, I.: Activation of Transcription by Guanosine 5'-Diphosphate, 3'-Diphosphate, Transfer RNA and a Novel Protein from E. Coli. J. Biol. Chem. 250: 2189-2195, 1975.

Pastan, I.: Regulation of Cellular Growth. In Metabolic Disorders. Proceedings of the Nobel Symposium, Stockholm, September 1974, Vol. 8, in press, 1975.

Nakanishi, S., Adhya, S., Gottesman, M., and Pastan, I.: Activation of Transcription at Specific Promoters by Glycerol. J. Biol. Chem. 249: 4050-4056, 1974.

Anderson, W.B., Gottesman, M.E., and Pastan, I.: Studies With the Cyclic Adenosine Monophosphate Receptor and Stimulation of In Vitro Transcription of the Gal Operon. J. Biol. Chem. 249: 3592-3596, 1974.

Pastan, I., Gallo, M., and Anderson, W.B.: The Purification and Analysis of Mechanism of Action of a Cyclic AMP-Receptor Protein from Escherichia Coli. In Hardman, J.G., O'Malley, B.W. (eds.): Methods in Enzymology-Hormone Action, Part C, Cyclic Nucleotides, 1974, pp. 367-376.

Serial No. Z01 CBD 8001-05 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Cyclic AMP in the Regulation of Growth and Morphology of Transformed and Normal Fibroblasts

Previous Serial Number: Same

Principal Investigator: Ira Pastan

Other Investigators: John Nesbitt, David Wallach, Zachary Miller, Jacques Pouyssegur, P. Thomopoulos, and Kenneth Olden

Cooperating Units: Clinical Endocrinology Branch, NIAMDD

Man Years: July 1, 1974 through June 30, 1975

Total:	6.8
Professional:	5.0
Others:	1.8

Project Description:

Objective: To understand the role of cyclic AMP in malignant transformation and how cyclic AMP regulates the growth, morphology, and other properties of cultured fibroblastic cells.

Methods Employed: Cell culture and standard biochemical analyses of the enzymes involved in cyclic AMP metabolism and the synthesis of macromolecules.

Major Findings: We have continued to characterize the adenylate cyclase (a.c.) of cultured mouse, rat and chick embryo fibroblasts. We have devised a convenient method to prepare large amounts of purified plasma membranes from NRK cells using gradient centrifugation. The a.c. of NRK cells is activated by prostaglandin E_1, epinephrine and GTP. Using detergents and proteolytic enzymes we can selectively block the activation by GTP or PGE_1. Further W. Anderson has found that the a.c. of NRK cells transformed by the Kirsten or Moloney viruses can be activated by GTP but not PGE_1. Thus PGE_1 and GTP agents must act by separate mechanisms.

J. Nesbitt has studied the guanylate cyclase (g.c.) of 3T3 and NRK cells. G.C. activity is low in growing cells and rises as their growth slows. Transformed 3T3 and NRK cells have markedly decreased g.c. and in Kirsten transformed NRK cells the g.c. activity is undetectable. KNRK cells also have very low or undetectable levels of cyclic GMP and some KNRK clones are also missing cGMP phosphodisterase. These findings are not in keeping with the widely held idea that cyclic GMP is an essential growth promoter.

Since insulin has been reported to promote the growth of cultured cells P. Thomopoulos has been measuring insulin binding using ^{125}I-insulin. He finds that the number of insulin binding sites are low in transformed 3T3 cells and in growing 3T3 cells but rise as 3T3 cells undergo density dependent inhibition of growth.

J. Pouyssegur has isolated mutants of Balb 3T3 cells that have the low adhesiveness characteristic of transformed cells but in other ways appear normal. He is using these mutant cells to identify the molecules that participate in the adhesive process.

Frequently untransformed cells are more sensitive to growth arrest by Bt_2cAMP than transformed derivatives. K. Olden and K. Cohn (NCI DCT) have been trying to exploit this differential sensitivity to protect selectively normal cells from killing by chemotherapeutic agents.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approaches 1, 2 and 5; Objective 4, Approach 2; Objective 6, Approach 3. Various aspects of this work will lead to a better understanding of how cells become cancer cells and how the growth of cancer cells is controlled. It also has therapeutic implications.

Proposed Course: 1. We will examine how the transformation event alters the ability of cells to maintain normal cyclic AMP levels. We also will study how cyclic AMP works to slow growth and produce its other effects. 2. We will investigate why some cells are much less responsive to cyclic AMP than others, and try to exploit this difference in chemotherapeutic trials.

Honors and Awards: None

Publications:

Willingham, M., and Pastan, I.: Cyclic AMP and Cell Morphology in Cultured Fibroblasts: Effects on Cell Shape, Microfilament and Microtubular Distribution, and Orientation to Substratum. J. Cell Biol., in press, 1975.

Pastan, I., Johnson, G., and Anderson, W.B.: Role of Cyclic Nucleotides in Growth Control. In Annual Review of Biochemistry. Vol. 44, in press, 1975.

Pastan, I.: The Role of Cyclic AMP in Malignant Transformation. Am. J. Clin. Path., in press, 1975.

Willingham, M.C., Ostlund, R.E., and Pastan, I.: Myosin is a Component of the Cell Surface of Cultured Cells. Proc. Nat. Acad. Sci. USA 30: 4144-4148, 1974.

Anderson, W.B., and Pastan, I.: Altered Adenylate Cyclase Activity: Its Role in Growth Regulation and Malignant Transformation of Fibroblasts. In Drummond, G.I., Greengard, P., and Robison, G.A. (eds.): Advances in Cyclic Nucleotide Research. New York, N.Y., Raven Press, 1975, Vol. 5.

Russell, T.R., and Pastan, I.: Cyclic Adenosine 3':5'-Monophosphate and Cyclic Guanosine 3':5'-Monophosphate Phosphodisterase Activities Are Under Separate Genetic Control. J. Biol. Chem. 249: 7764-7769, 1974.

Pastan, I.: Cyclic AMP and the Malignant Transformation of Cells. In Metabolic Disorders. Proceedings of the Nobel Symposium, Stockholm, September, 1974, Vol. 8, in press, 1975.

Anderson, W.B., Gallo, M., and Pastan, I.: Adenylate Cyclase Activity in Fibroblasts Transformed by Kirsten or Moloney Sarcoma Viruses: Decreased Activity and Loss of Response to Prostaglandin E_1. J. Biol. Chem. 249: 7041-7048, 1974.

Schwartz, J.P., Johnson, G.S., Pastan, I., and Passonneau, J.V.: The Effect of Growth Conditions on NAD^+ and NADH Concentrations and the NAD^+/NADH Ratio in Normal and Transformed Fibroblasts. J. Biol. Chem. 249: 4138-4143, 1974.

Key Word Descriptors: Cyclic AMP, Cyclic GMP, Transformation, Fibroblasts, Prostaglandins, Insulin, Growth

Serial No. Z01 CBD 8005-04 LMBGY
1. Laboratory of Molecular Biology
2. Biochemical Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Biochemistry of λ Int-Promoted Recombination

Previous Serial Number: Same

Princiapl Investigators: Susan Gottesman, Marc Shulman and Max E. Gottesman

Other Investigator: None

Cooperating Unit: None

Man Years: July 1, 1974 through June 30, 1975

Total:	2.7
Professional:	2.7
Others:	0.0

Project Description:

Objectives: Phage-induced functions promote the integration of λ DNA into the *E. coli* genome. Our objective is to study these functions at the genetic and biochemical level.

Methods Employed: Standard microbiological techniques and techniques developed for this project were used in this study.

Major Findings: (a) We have isolated λ with mutant attachment sites. The mutations reside in a region of homology between λ and *E. coli*. (b) The functions which promote integration cannot work sequentially. Translation, transcription, and replication are not required for the reaction. (c) An *in vitro* system in which λ prophage excision occurs has been established. The system requires the phage function Int and Xis, as well as ATP, Mg^{++} and spermidine.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 3. The biochemical basis of phage integration is at present completely unknown. The phage integration reaction will serve as a model system for studying the integration of oncogenic viruses in mammalian cells.

It provides information of potential value in determining the nature of oncogenic cell transformation and thus may ultimately permit the prevention of conversion of normal to tumorigenic cells.

Proposed Course: Project discontinued.

Honors and Awards: None

Publications:

Shulman, M., and Gottesman, M.E.: Attachment Site Mutants of Bacteriophage λ. *J. Mol. Biol.* 81: 461-483, 1973.

Gottesman, M.E.: The Integration and Excision of Bacteriophage Lambda. *Cell* 1: 69-72, 1974.

Shulman, M.: Model for Wandering Restriction Enzymes. *Nature* 252: 76, 1974.

Gottesman, S. and Gottesman, M.E.: The Elements Involved in Site-specific Recombination in Bacteriophage λ. *J. Mol. Biol.* 91: 489-499, 1975.

Gottesman, S. and Gottesman, M.E.: The Excision of Prophage λ in a Cell-free System. *Proc. Nat. Acad. Sci. U.S.A.*, in press.

Key Word Descriptors: Site-Specific Recombination, *In Vitro* Recombination, Bacterial Virus, Lambda

Serial No. Z01 CBD 8006-04 LMBGY
1. Laboratory of Molecular Biology
2. Biochemical Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control of Expression of the Galactose Operon in *E. coli* K12, Bacteriophage λ and P22

Previous Serial Number: Same

Principial Investigators: Sankar L. Adhya, Max E. Gottesman and Donald L. Court

Other Investigators: Benoit de Crombrugghe, Asis Das, Oscar Reyes and Sandra Hilliker

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	6.2
Professional:	6.0
Others:	0.2

Project Description:

Objectives: The enzymes of the galactose operon of *E. coli* are needed for utilization of galactose and normal cell morphology. The control of expression of the operon is complex. It is normally subjected to a negative control by *gal* repressor and a positive control by cyclic AMP. We have also shown that the galactose operon is and can be also subject to other control systems, including control by neighboring prophage genes. Our object is to determine the complete structure and to identify all the regulatory elements of the galactose operon of *E. coli*. These include the signals of initiation and termination of gene expression. We are also studying the expression of genes in bacteriophage λ and P22 growth.

Methods Employed: Standard microbial genetic and biochemical techniques.

Major Findings: The galactose operon is located close to the prophage λ on the chromosome of *E. coli*. It is known that induction of the prophage causes constitutive expression of the operon (escape synthesis). Last year we reported that one of the components of the escape synthesis is due to transcription originating in the prophage DNA and extending into *gal* operon. We have further shown that this phage promoted transcription is (i) not blocked by galactose repressor and (ii) not subject to normal cellular transcription termination mechanisms. The requirements of such termination resistant transcription have been defined. The resultant gal RNA transcript originating in a phage promoter is very large as expected, and unlike other cellular messenger RNA is extremely stable. This unusual stability is due to some phage gene(s). The relevant phage gene(s) is now being investigated.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Object 3, Approach 1. In cancer cells, the expression of some genes are permanently turned on, i.e. expressed constitutively. Our studies are aimed to understand the molecular basis of how genes are turned on and off. We are using gal as a model system. This understanding might help to prevent conversions of cells to those capable of forming cancers.

Proposed Course: 1. To determine the complete structure of the gal operon. 2. To identify all the regulatory molecules involved in turning on and off of the various genes of the gal operon, phage λ and P22. 3. To understand the biochemistry of the initiation and termination signals of gene expression.

Honors and Awards: None

Publications:

Adhya, S., Gottesman, M., and de Crombrugghe, B.: Release of Polarity in E. coli by gene N of Phage Lambda: Termination and Antitermination of Transcription. Proc. Nat. Acad. Sci. U.S.A. 71: 2534-2538, 1974.

Key Words: Regulation of gene expression, Virus-host interactions, Uncontrolled gene expression, Cyclic AMP, Gal operon, Transcription, Bacterial virus lambda

Serial No. Z01 CBD 8007-03 LMBGY
1. Laboratory of Molecular Biology
2. Biochemical Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Positive and Negative Regulations by Bacteriophage Gene Products

Previous Serial Number: Same

Principal Investigators: Donald L. Court and Max E. Gottesman

Other Investigators: None

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	1.1
Professional:	1.0
Others:	0.1

Project Description:

Objectives: We would like to define the events necessary for the establishment of lysogeny in a λ-infected cell. To do this, we have studied the cII and cIII functions of λ by genetic and biochemical approaches. These two λ functions are both known to be required for efficient λ lysogeny and λcI-repressor synthesis.

Methods Employed: Standard microbiological and biochemical techniques have been used in these studies.

Major Findings: cII and cIII mutants have been found to be deficient in the synthesis of cI repressor protein following infection and also to overproduce lytic gene proteins. cY mutants show this same phenotype but have been shown not to be mutant in a diffusible protein (like cII or cIII) but rather in a site. The action of cII and cIII function is proportional to the multiplicity of infecting cII^+cIII^+ phage.

A protein activity has been found in extracts of λcI^- infected cells that is able to bind specifically ^{32}P labelled λDNA to a membrane filter. Mutations in cII and cIII remove this activity.

SDS-acrylamido gel analysis using double labeled proteins reveals that ∿45,000 and ∿35,000 molecular weight peptides are lost when $\lambda\ cII^-$. These peptides are associated with the ribosomes and eluted with 0.5M NH_4Cl.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 3. As suggested recently by Huebner and Todaro (P.N.A.S. 64: 1087, 1969) a process analogous to lysogenization may be important in carcinogenesis. The relatively simple phage λ is a perfect model to use for an in vitro study of this process. As yet little is actually known about the regulation involved at the biochemical level for even the λ lysogenization system.

Proposed Course: This project has been discontinued.

Honors and Awards: None

Publications:

Court, D., Green, L. and Echols, H. Positive and Negative Regulation by the cII and cIII Gene Products of Bacteriophage Lambda. Virology 63: 484-491, 1975.

Ibid, Genetics (in preparation).

Key Words: Regulatory genes, Lysogeny, Bacterial virus, Lambda

Serial No. Z01 CBD 8008-03 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Initiation and Termination of Transcription of Bacterial Operons

Previous Serial Number: Same

Principal Investigator: Benoit de Crombrugghe

Other Investigators: Richard Musso, Bruce Howard, Roberto di Lauro, Martin Rosenberg

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	3.2
Professional:	3.0
Others:	0.2

Project Description:

Objective: To understand the precise biochemical mechanisms which control initiation and termination of transcription in *E. coli*.

Methods Employed: Measurement of RNA and protein synthesis *in vivo* and in cell-free systems; isolation of factors active in regulation of initiation and termination of transcription; isolation of specific DNA fragments; purification of specific RNA species; nucleotide sequence analysis of RNA and DNA.

Major Findings: (a) Transcription of the galactose operon of *E. coli* is known to be regulated by cyclic AMP and the cyclic AMP receptor and by an operon specific repressor. In addition, other regulatory factors are probably involved. We have used a kinetically controlled purified *in vitro* transcription system to determine the nucleotide sequence of the 5'-terminus of *gal* mRNA. The sequence is:

$\text{pppA-U-A}\ \boxed{\text{C-C-A-U}}\ \text{-A-}\ \boxed{\text{A-G}}\ \text{-C-}\ \boxed{\text{C-U}}\ \text{-A-}\ \boxed{\text{A-U-G-G-}}\ \text{-A-G-}$
10 20
C-G-A-A-U-U-A-U-G-A-G-A-G-U-U-C-U-G-G-U-U-A-C-C-G-G-U-G-
30 40
U-A-G-C-G-G-U-U-A-C-A-U-U-G-G-A-A-G-U-C-A-U-A-C-C-U-G-U
50 60 70

Residues 27-77 correspond to the amino terminal 17 amino acids of UDP galactose-4-epimerases, the protein encoded by the promoter-proximal structural gene of the operon. The sequence of residues 4-18 reflects a partial two-fold symmetry in the DNA centered at position 11. Twelve of these fifteen nucleotides can be base paired (indicated by boxes). The sequence of residues 22-33 shows a close homology (10 of 12 bases identical as underlined) with part of the lac operator sequence (Gilbert and Maxam, PNAS, 1973). Present studies include (1) purification of specific DNA segments containing part or all of the gal operator-promoter region; (2) determination of the nucleotide sequence of this region; (3) definition of the regulatory segments of this region; (4) analysis of the interaction between these DNA segments and the regulatory proteins controlling the gal operon.

(b) Termination of transcription occurs at defined DNA sequences located at the 3'-end of operons (or cistrons). With E. coli or bacteriophage DNA, the protein factor rho is required for proper termination by RNA polymerases at many of these sites. An RNA dependent nucleoside phosphohydrolase (ATPase) was found to be associated with rho (Lowery et al., 1974). Our experiments indicate that the hydrolysis of the β-γ phosphatate bond of a ribonucleoside triphosphate is required for rho mediated termination of transcription. Present studies aim at (1) defining the role of the ATPase activity in the transcriptional termination process. (2) Determining the nucleotide sequence around rho dependent termination sites in λDNA and gal DNA.

(c) The bacteriophage λ genome codes for a regulatory function (N) which controls transcription of phage RNA presumably by antagonizing the host transcription termination factor rho. The function will allow transcription to elongate beyond termination signals. Study of this phage promoted transcription should help in understanding the process of termination of transcription. A phage transcript was isolated and found to be (1) very large, and (2) unusually stable. Current studies aim at (1) understanding the basis for this abnormal stability, and (2) determining the precise role of N in antitermination.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 2, Approach 3. Studies on nucleotide sequences of the regulatory segments of DNA are just starting in several laboratories. Our aim is to obtain the nucleotide sequence of all the regulatory regions of the gal operon. These regions include the sites for binding of RNA polymerase, for initiation of transcription, for repressor binding, for action of termination factor(s). They will also include the ribosomal binding sites on gal mRNA. Information stemming from our studies as well as analogous studies on other bacterial operons will indicate the genetic code words for regulation of transcription. Precise understanding of gene expression in microorganisms should provide a basis for understanding how genes are controlled in normal and transformed cells.

Proposed Course: (1) Study the regulation of the galactose operon of E. coli by determining the nucleotide sequences of the regulatory segments of both wild type and mutant gal DNA, and by analyzing the interactions between the regulatory proteins and their DNA binding sites. (2) Search for additional factors which are active in the regulation of this operon, and determination of their mode of action. (3) Study the mechanisms which regulate termination of transcription in E. coli.

Honors and Awards: None

Publications:

Adhya, S., Gottesman, M., and de Crombrugghe, B.: Release of polarity in E. coli by gene N of phage lambda: Termination and antitermination of transcription. Proc. Nat. Acad. Sci. USA 71: 2534-2538, 1974.

Musso, R.E., de Crombrugghe, B., Pastan, I., Sklar, J., Yot, P., and Weissman, S.: The 5'-terminal nucleotide sequence of galactose messenger ribonucleic acid of Escherichia coli. Proc. Nat. Acad. Sci. USA 71: 4940-4944, 1974.

De Crombrugghe, B.: In Protein Synthesis. in press, 1975.

Rosenberg, M., de crombrugghe, B., and Weissman, S.: Termination of transcription in bacteriophage lambda: Heterogenous, 3'-terminal oligo-adenylate additions and the effect of Rho factor. J. Biol. Chem. in press, 1974.

Key Word Descriptors: Regulation of Gene Expression, Transcription, Regulation of Transcription, Protein Nucleic Interactions, Nucleotide Sequence Analysis

Serial No. Z01 CBD 8009-02 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Factors that Regulate Cyclic AMP Levels in Cultured Cells

Previous Serial Number: Same

Principal Investigator: George S. Johnson

Other Investigators: None

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	1.0
Professional:	1.0
Others:	0

Project Description:

Objectives: To determine which of the agents known to affect growth and morphology affect cyclic AMP levels and thus possibly exert their effects through cyclic AMP, and to determine if transformed cells are less susceptible to control by these agents.

Methods Employed: Standard cell culture and radioimmunoassay techniques will be used.

Major Findings: The addition of analogs of cyclic AMP or agents which elevate cyclic AMP levels results in alterations in cellular properties such as morphology, adhesion, motion, and growth. Since these agents make transformed cells phenotypically more like normal cells in the expression of these properties, an analysis of the mechanism of action of cyclic AMP would be useful for studying transformation.

The mechanism whereby cyclic AMP interacts within the cell is not clear but the use of agents which induce the same properties without changing cyclic AMP levels should help in the elucidation of this mechanism. Two such means have been discovered; addition of N^6 substituted adenine derivatives or the substitution of glucose in the growth medium by other sugars induces quickly and reversibly similar changes in cells but does not significantly alter cyclic AMP levels.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 6, Approach 3. By understanding better how growth is regulated in normal cells and how this regulation is altered by transformation, we may learn how to control the growth of certain cancer cells.

Proposed Course: The major findings suggest that cyclic AMP, N^6 substituted adenines, and sugars influence a central control locus. Attempts to understand this control region will be done. Cyclic GMP levels, Ca^{++} flux, and protein kinase activities will be studied.

Honors and Awards: None

Publications:

Johnson, G.S., D'Armiento, M., and Carchman, R.A.: N^6 substituted adenines induce cell elongation irrespective of the intracellular cyclic AMP levels. Experimental Cell Res. 85: 47-56, 1974.

Schwartz, J.P., Johnson, G.S., Pastan, I., and Passonneau, J.V.: The effect of growth conditions NAD^+ and NADH concentrations and the NAD^+/NADH ratio in normal and transformed fibroblasts. J. Biol. Chem. 249: 4138-4143, 1974.

Pastan, I., and Johnson, G.S.: Cyclic AMP and the transformation of fibroblasts. Advances in Cancer Research 19: 303-329, 1974.

Johnson, G.S.: Cyclic AMP and cellular transformation. In Nakahara, W., Ono, T., Sugimura, T., and Sugano, H. (eds.): Proceedings of the 4th International Symposium of the Princess Takamatsu Cancer Research Fund. Tokyo, Japan, University of Tokyo Press, 1974, pp. 89-98.

Johnson, G.S.: The importance of cyclic AMP in cellular transformation. In Cyclic Nucleotides in Disease, in press, 1975.

Pastan, I., Johnson, G., and Anderson, W.B.: Role of cyclic nucleotides in growth control. In Annual Review of Biochemistry 44: in press, 1975.

Keyword Descriptors: Cultured Cells, Growth Cells, Cell Morphology, Radioimmunoassay, Cyclic AMP, Glucose, Sugars

Serial No. Z01 CBD 8010-01 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Cyclic AMP in the Regulation of Morphology, Motility, Malignant Transformation, and Membrane-Mediated Phenomena in Cultured Cells

Previous Serial Number: None

Principal Investigator: Mark C. Willingham

Other Investigators: Ira H. Pastan and Ken Olden

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total: 1.4
Professional: 1.0
Others: 0.4

Project Description:

Objectives: To investigate the mechanisms that control the expression of properties of transformed cells in culture, with particular emphasis on the role of cyclic AMP in modulating these properties.

Methods Employed: Cell culture, standard biochemical analyses of cyclic nucleotides, specialized light and electron microscopic morphologic and immunohistochemical methods.

Major Findings: The past year has been devoted to studying the changes in cell motility, morphology, and lectin agglutination due to malignant transformation, and the effects of cyclic AMP on these properties.

In normal and transformed cultured cells, significant changes in cell shape and motility occur following the addition of cyclic AMP. High cyclic AMP levels are associated with flattened or spindly shape and lowered motility, whereas low levels result in cell rounding and rapid motility. Utilizing time-lapse phase microcinematography, we have observed an altered pattern of motility due to high cyclic AMP levels, with inhibition of retraction of extended cell processes. At the electron microscopic level, these shape and motility changes are accompanied by increased numbers of microtubules and bundles of 70 Å microfilaments. Using a newly developed method of evaluation of the orientation of cells to their substratum, we observed that high cyclic AMP levels cause cells to adhere more closely to their substratum, particularly under microfilament bundles.

Since contractile proteins such as myosin are likely to be involved in cell motility, we have studied the cellular localization of myosin in cultured cells. By immunohistochemical methods, we have found that myosin is a component of the plasma membrane, as well as being present in bundles of microfilaments in the cytoplasm.

Lectin agglutination is another property of transformed cells that is altered by cyclic AMP. We have found a quantitative rapid (15 min) decrease in agglutinability of transformed cells after cyclic AMP treatment, almost to the level of normal cells. By using dark field light and electron microscopy we have documented a rapid decrease in surface microvilli after cyclic AMP treatment that correlates with the changes in agglutination. We have accumulated evidence that increased agglutination of transformed cells by plant lectins is dependent on the presence of microvilli which are expressed due to the low cyclic AMP levels following malignant transformation.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 6, Approach 3. It appears that a major reason for the many changes in behavior of transformed cells in culture is their inability to maintain normal cyclic AMP levels. By understanding the properties regulated by cyclic AMP, or by raising cyclic AMP levels in these cells, one might control the spread or growth of certain types of cancer.

Proposed Course: We will study the mechanisms by which cyclic AMP exerts its normalizing effects on transformed cells and the role cyclic AMP may have in other properties altered in transformation.

Honors and Awards: None

Publications:

Pastan, I., Anderson, W.B., Carchman, R.A., Willingham, M.C., Russell, T.R., and Johnson, G.S.: Cyclic AMP and Malignant Transformation In Clarkson, B., and Baserga, R. (eds.): Control of Proliferation in Animal Cells. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1974, pp. 563-570.

Willingham, M.C., and Pastan, I.: Cyclic AMP mediates the concanavalin A agglutinability of mouse fibroblasts. J. Cell Biol. 63: 288-294, 1974.

Willingham, M.C., Ostlund, R.E., and Pastan, I.: Myosin is a component of the cell surface of cultured cells. Proc. Nat. Acad. Sci. USA 71: 4144-4148, 1974.

Willingham, M.C., and Pastan, I.: Cyclic AMP modulates microvillus formation and agglutinability in transformed and normal mouse fibroblasts. Proc. Nat. Acad. Sci. USA 1975, in press.

Willingham, M.C., and Pastan, I.: Cyclic AMP and cell morphology in cultured fibroblasts: Effects on cell shape, microfilament and microtubular distribution and orientation to substratum. J. Cell Biol. 1975, in press.

Willingham, M.C.: Cyclic AMP and the behavior of cultured cells. International Review of Cytology 1975, in press.

Key Words: Cyclic AMP, Malignant transformation, Motility, Cultured cell morphology, Cultured cell myosin, Lectin agglutination, Substratum adhesiveness, Plasma membrane antigens, Microfilaments and microtubules, Immunohistochemistry

Serial No. Z01 CBD 8011-01 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structure and Role of a Transformation-Sensitive Cell Surface Glycoprotein

Previous Serial Number: None

Principal Investigator: Kenneth M. Yamada

Other Investigators: Ira H. Pastan

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	1.2
Professional:	1.1
Others:	0.1

Project Description:

Objectives: A major cell surface protein (CSP) is depleted after viral transformation of fibroblasts *in vitro*. Our objectives are to determine CSP's biochemical structure and its role in cell behavior.

Methods Employed: CSP is isolated from chick embryo fibroblasts using urea, and quantitated on SDS polyacrylamide gels. Agglutinin activity of CSP preparations is measured by hemagglutination of serial dilutions. Effects of CSP on cell-cell adhesion are estimated using rotation-mediated aggregation of suspensions of single cells.

Major Findings: We had previously developed methods to isolate CSP and to reattach trace quantities to cultured cells. We have improved both the purity and recovery of CSP preparations several fold, and have obtained antibody to electrophoretically purified CSP. CSP preparations will agglutinate erythrocytes, and this activity is blocked by anti-CSP gamma globulin. Agglutination is also inhibited by trypsin and chelators such as EDTA. In contrast, extracts from transformed chick fibroblasts contain little CSP or hemagglutinating activity.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 5. Neoplastic cells are generally less adhesive. Our studies show that a major cell surface glycoprotein of fibroblasts, which is decreased after transformation, has adhesive properties. The role of such a cell surface component in normal versus transformed cell behavior can now be explored using a biologically active, isolated protein and its antiserum.

Proposed Course: To examine the distribution of CSP *in vitro* and *in vivo* using anti-CSP antibodies, the effects of exogenously supplied CSP on cell behavior, and the biochemical basis of its adhesiveness.

Honors and Awards: None

Publications:

Yamada, K.M.: Cell Morphogenetic Movements. In Wilson, J.G., and Fraser, F.C. (eds.): *Handbook of Teratology*. New York, Plenum Press, 1975, in press.

Key Word Descriptors: Cell Surface, Membrane Glycoprotein, Transformation, Cell Adhesion, Hemagglutination, Chick Embryo Fibroblasts, SDS Gels

Serial No. Z01 CBD 8012-01 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Adenylate Cyclase Activity of Transformed and Normal Fibroblasts

Previous Serial Number: None

Principal Investigator: Wayne B. Anderson

Other Investigators: Ira H. Pastan

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	3.1
Professional:	1.1
Others:	2.0

Project Description:

Objectives: To investigate how adenylate cyclase activity is regulated in normal fibroblasts and how the activity of this enzyme is altered with transformation of fibroblasts by RNA tumor viruses.

Methods Employed: Cell culture and standard biochemical analysis of adenylate cyclase activity.

Major Findings: Cyclic AMP has an important role in regulating many properties of normal fibroblasts, including cellular growth rate. Transformed fibroblasts have decreased cyclic AMP levels, and this decrease produces some of the abnormal properties of these cells.

Studies with several virally transformed fibroblast lines have indicated that it is a defect in adenylate cyclase which accounts for the low levels of cyclic AMP found in these cells. The means by which the different viruses alter the enzyme's activity appears to differ. It was found that the cyclase from Kirsten or Moloney sarcoma virus transformed rat kidney (NRK) cells has lost its responsiveness to activation by prostaglandin E_1 or epinephrine. Since adenylate cyclase is an integral component of the plasma membrane, modification of the membrane could alter either the catalytic or regulatory properties of the cyclase system.

We previously have shown that the specific activity of NRK adenylate cyclase increases with increasing cell population density.

Viral transformation apparently abolishes this response for the cyclase activity of transformed cells is low and rises only slightly with increasing cell density.

In studies to determine how normal cells might regulate adenylate cyclase, we found evidence for the existence of a soluble, macromolecular activator of this enzyme. While this activator is present in resting, confluent cells, it appears to be greatly diminished in sparse, rapidly proliferating NRK cells. Further, transformation may cause a decrease in the amount of this soluble activator or render adenylate cyclase unresponsive to stimulation by this factor.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 6, Approach 3. Employing several different RNA-containing sarcoma viruses we have found that a common feature of transformation is decreased adenylate cyclase activity with correspondingly low levels of cyclic AMP. In studying the altered enzymatic properties of adenylate cyclase we can learn something of the transformational events carried out by RNA tumor viruses. If it is possible to correct the defective enzyme of transformed cells, this would result in elevated cyclic AMP levels which in turn could revert some of the abnormal properties of cancer cells towards normal.

Proposed Course: (1) We will continue to elucidate the regulatory properties of the fibroblast adenylate cyclase system. Particularly, we will examine the nature of the supernatant activator and its possible role in regulating cellular growth. (2) We will investigate the biochemical nature of the changes in the plasma membrane caused by viral transformation which lead to altered adenylate cyclase activity.

Honors and Awards: None

Publications:

Anderson, W.B., Gallo, M., and Pastan, I.: Adenylate cyclase activity in fibroblasts transformed by Kirsten or Moloney sarcoma viruses. J. Biol. Chem. 249: 7041-7048, 1974.

Anderson, W.B., and Pastan, I.: Altered adenylate cyclase activity: Its role in growth regulation and malignant transformation of fibroblasts. In Drummond, G.I., Greengard, P., and Robison, G.A. (eds.): Advances in Cyclic Nucleotide Research. New York, Raven Press, 1975, Vol. 5, in press.

Pastan, I., Johnson, G., and Anderson, W.B.: Role of cyclic nucleotides in growth control. In Annual Review of Biochemistry. 1975, Vol. 44, in press.

Keyword Descriptors: Cyclic AMP, Adenylate Cyclase, Fibroblasts, RNA Tumor Viruses, Cell Growth Control

Serial No. Z01 CBD 8013-01 LMBGY
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An Examination of E. coli Polymerase Mediated Termination of Transcription and Post-transcriptional Processing of Certain RNA Species.

Previous Serial Number: None

Principal Investigator: Martin Rosenberg

Other Investigators: Benoit de Crombrugghe

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description:

Objectives: 1) to determine the nucleic acid structure involved in the control of transcriptional termination and post-transcriptional modification of RNA and to understand the functional relationship of this structure to the regulation of these processes.
2) to develop new techniques which can be applied to both these studies and to the general examination of nucleic acid structure in other biological systems.

Methods Employed: Purification of specific RNA transcripts synthesized either in vivo or in vitro; nucleic acid sequence analysis of both RNA and DNA; primed extension of RNA transcription products with deoxyribonucleotides; isolation of protein factors involved either in the processes of transcriptional termination or post-transcriptional modification of RNA.

Major Findings: 1) The nucleic acid primary structure at the termini of the bacteriophage T7 "early" mRNAs synthesized both in vivo and in vitro have been examined. The sequence information obtained supports a model for transcription of the "early" region in which a high molecular weight precursor RNA molecule is posttranscriptionally processed to yield monocistronic messengers. The processing agent is a host specified endonuclease, Ribonuclease III, which is also involved in the post-transcriptional processing of a ribosomal RNA precursor in *E. coli*. Thus, it was shown that the T7 "early" RNAs are direct products of RNase III cleavage of a large polycistronic precursor molecule.

In addition, we demonstrated that the 3'-termini of the processed RNAs contained sequence heterogeneity arising from the addition of several (up to 5) adenylate residues to the 3'-end. These A additions appear to be a non-template specified, post-transcriptional, post-processing, modification of the RNA.

In an effort to further elucidate the mode of action or RNase III in the processing event, as well as examine the entire structure of an intercistronic region of the phage, we have now isolated and are in the process of sequencing an RNA fragment which traverses the region between two adjacent "early" T7 genes (thereby spanning an RNase III cleavage site).

2) The nucleic acid structure of the 3'-terminal regions of a number of RNA transcripts synthesized *in vitro* from λ and λ *gal* transducing phages are being examined. These RNAs represent both independently terminated transcription products as well as transcripts requiring the protein factor, Rho, for their termination. Sequence analysis at the 3'-ends of some of these products has indicated:

a) that all of the transcripts examined possess similar degrees of 3'-terminal sequence hetereogeneity which consisted predominately of the addition of 1 to 5 adenylate residues to the 3'-terminus of the transcript and b) that rho factor enhanced termination results in a definite structural change in the nucleotide sequence with which an RNA molecule can terminate.

In addition, we have developed a new methodology for examining the nucleic acid structure in the untranscribed portion of the DNA immediately adjacent to the 3'-terminus of an RNA transcript. This technique is being applied to the determination of sequence information throughout the entire termination region of these transcription products.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 1. The regulation of gene expression in both prokaryotic and eukaryotic systems involves numerous and complex structural and functional interactions between nucleic acids and proteins. It is highly probable (and experimental evidence increasingly supports the contention) that many of the nucleic acid fine-structure interactions which occur on the prokaryotic level have basic similarities to analogous type interactions known to occur in higher cells. Our studies involve the determination of the nucleic acid structure involved in the control of the termination of transcription by RNA polymerase and the post-transcriptional processing of certain RNA species by specific endonucleases (e.g. RNase III) in both bacterial and related viral systems. The elucidation of these transcriptional processes and their regulatory elements will be a basis for understanding similar processes occurring in eukaryotic cells (both normal and transformed) and virus (oncogenic and other).

Proposed Course: To examine further the mechanisms by which RNA transcripts are terminated and subsequently processed during the bacterial and viral life cycle.

Honors and Awards: None

Publications:

Rosenberg, M.: Isolation and sequence determination of the 3'-terminal regions of isotopically labelled RNA molecules. Nucleic Acids Research 1: 653-671, 1974.

Rosenberg, M., Kramer, R.A., and Steitz, J.A.: T7 early messenger RNAs are the direct products of ribonuclease III cleavage. J. Mol. Biol. 89: 777-782, 1974.

Kramer, R.A., Rosenberg, M., and Steitz, J.A.: Nucleotide sequences of the 5'- and 3'-termini of bacteriophage T7 early messenger RNAs synthesized in vivo: Evidence for sequence specificity in RNA processing. J. Mol. Biol. 89: 767-776, 1974.

Rosenberg, M., Kramer, R.A., and Steitz, J.A.: The specificity of RNase III cleavage of bacteriophage T7 early messenger RNAs. Brookhaven Symp. in Biol. 26: 1975, in press.

Rosenberg, M., de Crombrugghe, B., and Weissman, S.: Termination of transcription in bacteriophage lambda: Heterogeneous, 3'-terminal oligo-adenylate additions and the effect of rho factor. J. Biol. Chem., 1975, in press.

Key Words: Nucleic acid sequence analysis, RNA transcription, Hybridization, RNA processing, RNase III, Termination of transcription, Rho factor

Serial No. Z01 CBD 905-23 LMBGY
1. Laboratory of Molecular Biology
2. Cell Organization Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Thyroid Hormone Synthesis and Storage

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: Ragnar Ekholm, Andre Haeberli, and Lois Tice

Cooperating Units: Laboratory of Experimental Pathology, National Institute of Arthritis & Metabolic Diseases, NIH, Bethesda, Md. (Serial No. NIAMD, LEP-10)

Man Years: July 1, 1974 through June 30, 1975

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To learn about the mechanism of synthesis and storage of thyroid hormones.

Methods Employed: Localization of radioiodoprotein at short time intervals after injection of radioiodide: autoradiographs were prepared of 0.1 μm sections of tissues embedded in Epon and they were examined by electron microscopy. For localization of Calcium-45: thyroids were removed at various time intervals after injection of the ^{45}Ca and immediately frozen. Frozen sections were cut and autoradiographs prepared of the sections while frozen.

Major Findings:

Site of iodination of thyroglobulin (with Dr. Ekholm): The location of newly formed iodoprotein (within 30 seconds after injection of radioiodine) was determined using electron microscope autoradiography. At this time essentially all radioisotope was in the lumen of the thyroid follicle. Since the transit time of thyroglobulin from the various intracellular organelles to lumen is much longer than 30 seconds the results indicate that iodination is an extracellular event.

Calcium (with Dr. Haeberli): The location of calcium-45 in the thyroid gland was ascertained by autoradiography of frozen sections of the thyroid glands of rats injected with radiocalcium. Ten minutes and one hour after injection most of ^{45}Ca is in the cell but by 24 hours the concentration in the lumen is higher than in the cell and in the blood. The interaction of calcium with thyroglobulin was investigated. It was found that at the concentrations present in the lumen of the follicle calcium markedly increases the viscosity of thyroglobulin solutions and at slightly higher concentrations causes gelling. Significant elevation of free calcium must occur in thyroid cells for short periods after macropinocytosis of thyroglobulin from the lumen.

Proposed Course: Further work is planned on the gelling of thyroglobulin in the presence of calcium and possibly on the effect on the cell of calcium ingested during pinocytosis.

Significance for Cancer Research and the Program of the Institute: Some of the work reported here may best be put into Objective 3, Approach 5 because they give information on properties of cell surface and cell membranes.

Honors and Awards: None

Publication:

Tice, L.W. and Wollman, S.H.: Ultrastructural Localization of Peroxidase on Pseudopods and Other Structures of the Typical Thyroid Epithelial Cell. Endocrinology 94: 1555-1567, 1974.

Key Words: Iodination, Radioisotopes, Calcium, Thyroglobulin, Cell junctions, Autoradiography, Ultrastructure, Plasma membrane, Thyroid gland

Serial No. Z01 CBD 906-23 LMBGY
1. Laboratory of Molecular Biology
2. Cell Organization Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Production and Properties of Transplantable Thyroid Tumors

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: J. D. Zeligs and S. R. Hilfer

Cooperating Units: Department of Biology, Temple University, Philadelphia, Pennsylvania

Man Years: July 1, 1974 through June 30, 1975

Total: 1.0
Professional: 1.0
Others: 0

Project Description:

Objectives: To study thyroid growth and the production and properties of transplantable thyroid tumors in the rat: changes occurring in the thyroid during growth, hyperplasia and involution; histologic types of thyroid tumors and their progression, and the correlation of histology with the biochemistry and physiology of the tumors. We are also studying a variety of cells recently recognized to be present in the normal thyroid gland and which may be the cells of origin of some of the different histologic types of thyroid tumors observed in the past.

Methods Employed: Origin of cell types in thyroid: ventral and ultimobranchial outpocketings of the embryonic pharynx were removed prior to their fusion to form the developing thyroid gland. They were implanted separately in the kidney capsule of the adult and allowed to differentiate. Later they were removed and examined by electron microscopy. Hyperplasia: Hyperplasia was produced in rat thyroid gland by feeding of the goitrogen, thiouracil. Various details of the nature of the growth process were examined by electron microscopy. Details included the nature of the mitotic process in epithelial and endothelial cells.

Major Findings: Origin of various cell types (with Dr. Hilfer): During embryologic development ultimobranchial bodies (lateral outpocketings from the pharynx) fuse with a ventral outpocketing ultimately producing the thyroid gland. It was reported last year that if the ventral outpocketing is transplanted (before fusion) to kidney capsule it produces typical thyroid epithelium. It is now clear that if the lateral outpocketing in the C3H mouse is transplanted (before fusion) to kidney capsule it yields at least 4 different cell types. These include a ciliated cell, a cell with an abundant smooth endoplasmic reticulum, a cell with much free ribosome and clusters of fibrils, and the C-cell which produces calcitonin. In the adult gland these cells are found in a second type of thyroid follicle.

Mitosis in the hyperplastic thyroid gland (with Dr. Zeligs): As part of a study of thyroid hyperplasia, observations have been made of ultrastructural features of the typical thyroid epithelial cell during the mitotic cycle. Although there are many reports of observations of mitosis of cells in tissue culture there are only limited ultrastructure reports on the mitosis of cells in vivo and in particular of polarized cells. We have found that mitotic cells maintain contact with both lumen and basal lamina. Junctional complexes with neighboring cells remain intact. The mitotic spindle is oriented with axis parallel to lumen surface. The intracellular organelles all seem to persist through mitosis although some such as the rough endoplasmic reticulum change their position and structure radically.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan Objective 3, Approach 1. The properties of cells and of the tumors derived from them frequently depend upon the embryologic origin of the cells. This project will allow us to understand better the properties of certain tumors. The study of thyroid hyperplasia may give insight into conditions existing in the gland at the time that the neoplastic transformation occurs.

Proposed Course: I plan to continue the studies of hyperplasia. One study just initiated involves trying to do quantitative morphologic studies of changes in size of blood vessels, and of changes in relative abundance of the various cell types in the gland during growth.

Honors and Awards: None

Publications: None

Key Words: Embryology, Mitosis, Ultrastructure, Hyperplasia, Thyroid gland, Involution, Cell death, Endothelium, Thyroid tumors

Serial No. Z01 CBD 8401-14 LMBGY
1. Laboratory of Molecular Biology
2. Cell Organization Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Endocytosis in the Thyroid Gland

Previous Serial Number: NCI-928

Principal Investigator: Seymour H. Wollman

Other Investigator: Joseph D. Zeligs

Cooperating Units: None

Man Years: July 1, 1974 through June 30, 1975

Total:	1.5
Professional:	1.0
Others:	0.5

Project Description:

Objectives: To determine mechanism of thyroid hormone release, and more generally, endocytotic properties of typical thyroid epithelium.

Methods Employed: During the course of production of thyroid hyperplasia by the feeding of thiouracil microhemorrhages occur in the thyroid gland. The stimulated epithelial cells also take up stored thyroglobulin by macropinocytosis. Both of these phenomena are studied at the ultrastructural level by electron microscopy.

Major Findings: The typical thyroid epithelial cell can phagocytize red cells and cell debris from the lumen of the follicle. The process appears somewhat different in detail from macropinocytosis. The observations suggest that the epithelial cell has receptors for activation of pseudopod induction on the apical plasma membrane in addition to receptors at other sites sensitive to TSH.

The thyroid epithelial cell does not take up colloid by macropinocytosis in the very hyperplastic gland. Our investigations show that the cell is still responsive and produces early stages at the macropinocytotic process, but that the pseudopods do not mature and engulf colloid.

Significance for Cancer Research and the Program of the Institute: This project fits most closely under Objective 3, Approach 5, having to do with properties of the cell surface.

Proposed Course: Further studies of the nature of these processes will be undertaken, especially the fate of ingested red cells and the reason why intracellular colloid droplets are not formed after macropinocytosis is initiated.

Honors and Awards: None

Publications:

Bernstein, L.H. and Wollman, S.H.: Association of Mitochondria with Desmosomes in the Rat Thyroid Gland. J. Supramolecular Structure, in press, 1975.

Key Words: Pinocytosis, Phagocytosis, Ultrastructure, Thyroid gland,
Xysosomes, Pseudopods, Autoradiography

SUMMARY REPORT

LABORATORY OF PHYSIOLOGY

July 1, 1974, Through June 30, 1975

The scope of the research in the Laboratory of Physiology was narrowed somewhat by the departure of Dr. S. H. Wollman, who transferred to another Laboratory. In general, the following grouping of research thrusts can be construed: (1) Metabolism studies of the regulation and energy expenditures as well as the interdependence of metabolic and behavioral responses; (2) Control of the synthesis of proteins and nucleii acids; (3) Molecular and biological effects of radiation; and (4) Search for agents with antitumor activity.

The objectives of the metabolism studies covered investigation of how the energy expenditure, feeding and general behavior pattern are related to tumor growth, studies of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth, and development of quantitative and reproducible assessment of behavioral output, with hope of detecting early systemic effects of tumors, as well as searching for experimental models for cachexia and decline in food intake observed during neoplastic growth.

Change in diurnal variation of feeding and motor activity in cachectic tumor-bearers is similar to change in diurnal variation induced by chronic hypothalamic damage. This is the first evidence of a connection between the cachectic process and dysfunction of central control of feeding. Feeding responses to nutrient density, environmental temperature and exogenous insulin are distorted by tumor growth. The precise patterns of distortion are complex. In general, feeding responses of tumor-bearers to feeding stimuli are attenuated, but there appears to be substantial dependence on the effectiveness of the precancerous control of feeding of the host. Changes in feeding pattern in tumor growth and the extent to which food intake is modified by change in amount of feeding activity or change in feeding efficiency are being examined with respect to various normal influences on food intake and to changes with tumor growth and brain damage, separately and in combination.

To discover if there are differences in the structure, properties and metabolism of the nucleii acids and nucleoproteins of normal tissue in comparison to tissue becoming neoplastic, investigation of nucleotide metabolism in normal and cancer cells was carried out. In mammalian tissues, uridine-cytidine kinase is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction is rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end products. This enzyme was purified 1800-fold from the mouse ascites mast cell neoplasm P815/S and characterized with respect to stability, pH optimum, divalent cation requirement, and substrate specificity. The enzyme exhibited a broad specificity for the phosphate donor substrate, but a lesser one for the phosphate acceptor substrate. The ratio of uridine to cytidine kinase activity remained constant throughout the purification of the enzyme. The purified enzyme has a molecular weight of at least 150,000.

The UMP-CMP kinase has been purified 300-fold from Tetrahymena pyriformis, a rapidly growing protozoan unable to synthesize the pyrimidine ring de novo and hence dependent on anabolism from the preformed ring. The purified Tetrahymena enzyme also retains capacity to phosphorylate dCMP. This is the first extensive purification for CMP-UMP kinase activity; this enzyme has now been renumbered as EC 2.7.4.14, ATP: (d)CMP phosphotransferase. This purified enzyme was most stable in phosphate buffer, had a pH optimum between 7.0 and 8.0, required Mg^{2+}, was inhibited by CDP, UDP, ADP, and high levels of ATP.Mg. Subsequent studies revealed that the mechanism of the enzymatic reaction was probably sequential by addition of both substrates to the enzyme to form a ternary complex. Although the reaction is freely reversible, physiologically the enzyme is intended to function primarily in the forward or anabolic direction. The sequential addition of CMP and $ATP.Mg^{2-}$ to the enzyme occurs in a random fashion.

Since the replication of DNA is an obligatory prerequisite for cell division, and since the malignant cells display continuous and uncontrolled division, the study of DNA synthesis and its control occupies a place of unique importance in cancer research. One of the objectives is pursued by studying differences between normal and malignant cells that can be exploited to prevent the formation of cancer cells or to promote the destruction of cancer cells already generated. Considerable developmental work on methodology has been carried out in an attempt to improve ways for isolating and identifying deoxyribose-containing compounds other than DNA from normal, precancerous and tumor tissues, for isolating DNA from subcellular structures other than nuclei.

Whole-body irradiation in mice leaves a small amount of irreparable damage which reduces tolerance to a subsequent exposure. In leg-shielded animals no such residual damage can be seen. It appears that this damage is associated with the bone marrow rather than with the gastrointestinal tract.

Studies continue on immunogenetic factors influencing bone marrow transplantation in mice, with the goal of avoiding GVH reaction, so that this procedure can become an adjunct to heavy radiation or chemotherapy in the treatment of cancer. Marrow from the mother produces a less severe reaction in radiated mouse progeny than that from the father, presumably because of the persistence of tolerance for antigenic differences between mother and fetus. It was also noted that radiated daughter mice were more tolerant of mothers' marrow than radiated sons; this was true both for inbred and hybrid strains, suggesting that it is due to antigens controlled by the Y chromosome. These phenomena have now been studied by transplantation of fertilized ova of one strain into foster mothers of another. The results generally confirm the idea that phenotypic expression of the antigens of the fetus, and responsiveness as well, is reduced by maternal influences.

Research continues on the effects of ionizing radiation on nucleic acids, proteins and their constituents. Radioprotective and radiosensitizing agents are being studied in order to develop new approaches to increase the differential radiation sensitivity of tumors. In the chain of events that lead to loss of biological activity, free radicals play an important role. Recently, it was found that addition of certain metal ions to DNA at a 1:100 ratio of metal ions:nucleotides can reduce the radical yield by a factor of 10. From the ESR studies of cupric-DNA complexes, it was inferred that cupric ions compete with the bases in DNA for electrons produced by X-radiation.

Study of the effect of visible light on DNA in solid state, previously irradiated in the dark at 77°K showed a 75% drop of the ESR signal, suggesting that ionic radicals produced by X-radiolysis at 77°K in DNA and its constituents are light sensitive.

Earlier experiments have shown that some of the β-hydroxy-amino acids exhibited growth-inhibitory activity in microbial systems selected for antitumor screening and probable tumor-inhibitory action in some mammalian systems. Four isomers of N-chloroacetyl-β-hydroxynorleucine have been prepared in quantities permitting minimal testing in three mammalian systems. Microbial screening tests for antitumor action showed that non-acylated compounds were not growth-inhibitory. On the other hand, N-chloroacetylated derivatives show modest but significant antitumor activity. In light of this, N-acyl derivatives of other amino acids were prepared: Chloroacetyl-DL-allylglycine, chloroacetyl-DL-β-2-thienylalanine and chloroacetyl-DL-p-fluorophenylalanine.

Among the new projects just initiated, analogues of known chemotherapeutic agents are synthesized with the hope of intensifying the potency and lowering general toxicity. Nutritional status of tumor cells will be varied looking for conditions interfering with metabolic inactivation of chemotherapeutic agents. Finally, studies will be initiated on modification of cell membrane of susceptible and resistant tumor cells attempting to modify the permeability and susceptibility to chemotherapeutic agents.

Project No. Z01 CBD 08400-01 LPHY
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of Strategies for Potentiation of Chemotherapeutic Activities

Previous Serial Number: NCI-925 has been terminated.
Protein Synthesis and Its Control in Normal and Tumor Cells

Principal Investigator: Marco Rabinovitz

Other Investigator: Dr. Saburo Ayukawa, Visiting Fellow until March 1975

Man Years:

Total:	3 3/4
Professional:	1 3/4
Others:	2

Project Descriptions:

Objectives: To investigate the mechanism of action of selected chemotherapeutic agents, and develop means to promote their potency and specificity. Approaches to these goals will be attempted by a combination of three routes.

(1) Synthesis of analogs of known agents will be performed, in order to introduce modifications which should make them either more effective or of lesser general toxicity.

(2) Alteration of the nutritional status of tumor cells will be carried out, both in culture or *in vivo*, to produce conditions interfering with the metabolic inactivation of the chemotherapeutic agent. This approach will be particularly directed against resistant cell varieties and will be carried out by introduction of deficiencies accompanied with available or newly synthesized antimetabolites.

(3) Modification of the cell membrane of susceptible and resistant varieties of tumor cells will be attempted with the use of either available or newly synthesized membrane-reactive agents. Studies will be performed to estimate their effects on permeability and susceptibility to chemotherapeutic agents.

Major Findings: There have been no major findings in the currently initiated project. Laboratory activities have been directed toward establishing synthetic and assay procedures.

Significance for Cancer Research and the Program of the Institute: These studies fall under Objective 6, Approaches 1 and 2 of the National Cancer Plan, --"to develop and utilize methodologies of * * chemotherapy * * in the cure and palliation of cancer and to discover new therapeutic agents and develop means for their selection."

Keyword Descriptors:

Chemotherapeutic, potentiation, chemotherapy, cellular resistance, antimetabolites, membrane-reactive agents

Publications:

Rabinovitz, M: Translational repression in the control of globin chain initiation by hemin. Ann. N.Y. Acad. Sci. 241: 322-333, 1974.

Project No. Z01 CBD 00380-19 LPHY
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Deoxyribose containing compounds in normal, regenerating and cancer tissues

Previous Serial Number: Same

Principal Investigator: Walter C. Schneider

Man Years:

Total:	3.5
Professional:	1.0
Others:	2.5

Project Description:

Objectives: Since the replication of DNA is an obligatory prerequisite for cell division, the study of DNA synthesis and its control occupies a position of unique importance in cancer research because the malignant cancer cell displays continuous and uncontrolled division. The objectives of this project are to isolate and identify deoxyribose-containing compounds other than DNA from normal, precancerous, and tumor tissues, to determine the metabolic sequences in which these compounds are involved and whether these sequences are the same in normal precancerous and cancer tissues; to isolate DNA from subcellular structures other than nuclei, and to study the physical and biochemical properties of extranuclear DNA and to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

These studies are aimed at uncovering differences between normal and malignant cells that can be exploited to prevent the formation of cancer cells or to promote the destruction of cancer cells already generated. (These studies support most closely Objective 3: To prevent conversion of cells to those capable of forming cancer and Approach 1: Nature and modification of the precancerous state, of the National Cancer Plan).

Methods Employed: Nuclei, mitochondria, zymogen granules, and microsomes were isolated from rat or mouse tissues by differential and/or density gradient centrifugation as well as by centrifugation in the zonal rotor. Nucleic acids were extracted from tissue fractions using sodium lauryl sulfate (SLS) and phenol. DNA was purified from these extracts by treatment with ribonuclease and passage through Biogel P-60, by banding in CsCl, by chromatography on hydroxylapatite, or by treatment with NaOH to remove RNA and small molecules.

DNA samples were examined by means of analytical CsCl density gradient centrifugation, melting behavior, and amount of labeled thymidine incorporated. DNA was measured by a modification of the diphenylamine reaction. Enzyme markers were used for estimating purity of isolated subcellular fractions.

Major Findings: Although this first year in Building 10 was marked by a number of interruptions and delays due to renovations and the absence of essential services such as a fume hood and distilled water in several of the laboratories, most of these deficiencies were rectified before the end of the year and a considerable amount of work was accomplished. Among these accomplishments was the completion of three manuscripts: a revision of a series of tables of enzymatic activities of isolated subcellular organelles for the Handbook of Biochemistry, a paper on the separation of mitochondrial and microsomal DNA with detergents, and a manuscript on DNA associated with melanin granules in the S-91 melanoma and Amphiuma liver.

In addition, a considerable amount of time and effort was spent on new methods to facilitate future research. One was the development of the preparative ultracentrifuge as a substitute for the analytical ultracentrifuge (no longer available to us) in analyzing DNA samples by density gradient centrifugation in CsCl. Through the use of an Isco monitor and tube fractionator it was possible to obtain patterns for nuclear, mitochondrial, and microsomal DNA in mixtures with a marker DNA. The technique was considerably limited as compared to analytical ultracentrifugation, however, since the patterns could be observed only at the completion of centrifugation and also because scanning of each pattern required 30-45 minutes. The patterns in the preparative centrifuge were also not as sharp as those seen previously in the analytical machine. The preparative runs are further complicated by the geometry of the centrifuge tube, which makes assignment of densities to unknown peaks difficult despite the presence of marker DNA samples.

Attempts to provide better and more rapid measurements of DNA in tissue samples through fluoresence in the presence of ethidium bromide or acridine orange and specific enzyme digestion with deoxyribonuclease or ribonuclease but the results were not encouraging.

Attempts to use the Tissuemizer homogenizer in place of the Potter Elvejhem homogenizer were not successful. Although the former produced homogenates whose subcellular components were beautiful when examined with the phase microscope, mitochondria and microsomes isolated from such homogenates were contaminated with nuclear DNA and also were difficult to separate from each other.

Attempts to simplify the isolation of DNA from tissue fractions have had some success. In the usual procedure the sample is lysed with SLS, equilibrated with phenol and after centrifugation, the aqueous phase is mixed with ethanol to precipitate the nucleic acids. We have recently found that DNA can be banded in CsCl directly by lysing the fractions in sodium lauroyl sarcosinate and centrifugation for 48-66 hours. Proteins form an insoluble raft at the top of the tube and RNA, a pellet on the bottom. The costs in terms of CsCl and time of centrifuging are rather high for this method. A simpler, more rapid and cheaper approach was found by lysing fractions in SLS, adding NaCl to 1M and spinning at 20,000 rpm to sediment protein-SLS complexes. Protein determinations indicate that 90% or more of the original protein in each fraction could be removed in this way. Final purification of the DNA can then be performed by chromatography on hydroxylapatite. Although DNA can be isolated from fractions of nuclei, mitochondria, and microsomes by direct chromatography on hydroxylapatite, this has usually been fraught with technical difficulties due to shrinking and clogging of the columns.

Three new approaches are being tried to differentiate microsomal from nuclear DNA. One is to use mouse liver rather than rat liver as a tissue source since its nuclei contain satellite DNA. Our previous work with mouse liver microsomes showed a small peak of satellite DNA whereas Bond and his coworkers reported that no satellite was present in mouse microsomal DNA; therefore it appeared important to reinvestigate this point. In one new experiment using mouse microsomal DNA isolated by the new detergent procedure, no satellite DNA was detected in scans of CsCl gradients produced in the preparative ultracentrifuge although nuclear DNA showed definite satellite peaks.

Recent reports have appeared indicating that mitochondrial DNA was devoid of methyl cytosine and suggested that perhaps microsomal DNA could also be differentiated on this basis. Experiments with Dr. Ray Gantt have been begun to test this point using high pressure liquid chromatographic separation of the bases from DNA hydrolysates.

Finally, the reassociation kinetics of denatured DNA samples are being reinvestigated using the hydroxylapatite column method described in 1974 by Britten. Although Miss Greco has previously used an adaptation of this method in attempts to differentiate microsomal from other types of DNA, the results have been difficult to reproduce. It is hoped that the newer method will yield more positive results.

Significance for Cancer Research and the Program of the Institute: The results reported add to our basic store of knowledge about cytoplasmic DNA and to the methodology used in their study. The experiments were directed mainly at establishing whether microsomal DNA can be differentiated from nuclear DNA, an important first step that must precede the study of microsomal DNA in tumors or during the precancerous process. Since the experiments dealt with normal liver, they most closely support Objective 3, Approach 1 of the National Cancer Plan. Little or no work has been done on cytoplasmic DNA in tumors, or during growth or carcinogenesis and since the biological function of microsomal and mitochondrial DNA remains to be established, critical evaluation of these nucleic acids in the carcinogenic process must await further work.

Proposed Course of Research: With the aid of reassociation kinetics studies, analyses of base composition, and search for satellite DNA, it is hoped that an independent parameter can be added, other than rate of thymidine-^{3}H incorporation, to distinguish microsomal from nuclear and mitochondrial DNA. Then it will be possible to go on and study these cytoplasmic DNAs in other biological systems to learn their function. The study of DNA in zymogen granules is another project that needs to be completed; the basic groundwork for doing so has already been laid.

Keyword Descriptors:

Deoxyribonucleic acid, nuclei, mitochondria, microsomes, rodent liver, DNA reassociation kinetics, DNA base composition, cytoplasmic DNA, density gradient fractionation of DNA, satellite DNA

Publications:

Schneider, W. C.: Cytoplasmic DNA: Separation into two components with detergents. Biochem. Biophys. Res. Comm. 62: 492-496, 1975.

Schneider, W. C.: Enzymatic activities of subcellular fractions. In Fasman, G. D. (Ed.): Handbook of Biochemistry and Molecular Biology. Cleveland, Ohio, Chemical Rubber Co. (in press)

Project No. Z01 CBD 05221-02 LPHY
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies comparing the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with comparable tissues that become neoplastic

Previous Serial Number: Same

Principal Investigator: Jean Rotherham

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To discover if there are differences in the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with respect to comparable tissues that are becoming neoplastic.

Methods Employed: Suitable chemical agents will be used which enhance the occurrence of neoplastic changes in mouse and/or rat tissues. Nuclear fractions will be isolated and examined for possible differences in metabolic modifications of the nucleoproteins of tissues of treated and control animals.

Major Findings: For one series of experiments crude nuclear preparations from epidermis of both control and treated mice have been obtained. From each of these preparations a protein fraction was isolated, purified, and separated into component sub-groups by methods which have been used for resolution of similar protein fractions obtained from animal tissues other than epidermis. The amount of protein within each sub-group was determined with moderately good accuracy. Radiochemical measurements have been made on the unfractionated purified protein.

Based upon the radiochemical determinations it appears that, at one of the selected time intervals following application of croton oil to the epidermis, there was a biochemical difference between the protein obtained from untreated animals as compared with that from treated animals. Presumably this difference is the result of an effect of the "tumor promoter" on cell metabolism, possibly on the synthesis or metabolic modification of this group of proteins.

It should be noted that this observation would be more meaningful if confirmed by repetition and if the possibility of contaminating impurities were further reduced and the exact chemical nature of the apparent modification were determined. If a difference in radiochemical content of one or more of the component protein sub-groups from the two groups of animals were observed the possibility that this difference was due to contaminating impurities would be considerably less. Also by selecting the indicated appropriate time interval, attempting to obtain cleaner nuclear preparations with larger amounts of starting material and by chemical analysis of the modified protein fraction, the chemical nature of the apparent modification could be more clearly defined.

Significance for Cancer Research: (See objectives). National Cancer Program Plan Objective and Approach: Cause and prevention; nature and rate of oncogenic cell transformations in carcinogenesis (3, 2).

Proposed Course of Research: An attempt will be made to confirm these findings and to obtain an amount of the purified protein fractions sufficient for chemical analysis.

Keyword Descriptors:

Croton oil, nucleoproteins

Project No. Z01 CBD 05205-08 LPHY
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on biochemical mechanisms of sensitivity and resistance to antimetabolites and mechanisms for control of nucleic acid synthesis

Previous Serial Number: Same

Principal Investigator: Elizabeth P. Anderson

Other Investigators: Alec S. Liacouras

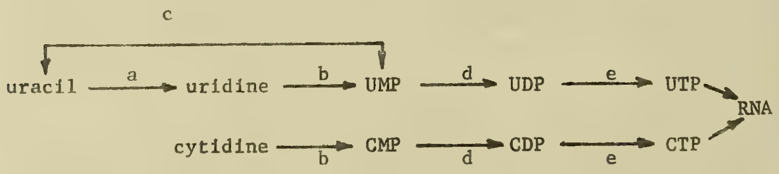
Cooperating Units: F. K. Millar, Laboratory of Theoretical Biology

Man Years:

Total:	2 + 1/2 (STRIDE)
Professional:	1
Others:	1 + 1/2 (STRIDE)

Project Description:

Objectives: The objective of this project is to investigate nucleotide metabolism in normal and cancer cells. The aim is to elucidate pathways of biosynthesis, mechanisms of control over these pathways, and biochemical mechanisms of sensitivity and resistance to purine and pyrimidine analogs. The end-products of nucleotide biosynthesis are the triphosphate precursors of nucleic acids, and regulation of the enzymes involved in these biosynthetic steps is probably a major factor in determining the rate of cellular nucleic acid synthesis. In view of the central role of UMP in pyrimidine metabolism, work has been focussed on reactions b and d in the following pathway of pyrimidine nucleotide biosynthesis:



Methods Employed and Major Findings:

1) Studies on uridine-cytidine kinase. In mammalian tissues uridine-cytidine kinase (reaction b above) is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction has been shown to be rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end-products (Anderson et al., Biochim. Biophys. Acta 91: 380, 1964). We have now purified this enzyme from the mouse ascites mast cell neoplasm P815/S, in which we first demonstrated feedback regulation of the enzyme. The purification procedure includes chromatography on DEAE-cellulose, ammonium sulfate fractionation, and separation on hydroxylapatite. The final preparation has a specific activity of 3.02 u/mg, the most highly purified preparation of this enzyme yet isolated; this represents 1300-fold purification from the whole tumor tissue. The final fraction is essentially free of UMP kinase, nucleoside diphosphate kinase, cytidine deaminase, adenosine triphosphatase, and enzymes which degrade UMP to uracil or uridine. We have characterized the purified enzyme with respect to stability, requirements of the enzymatic reaction, including divalent cation requirement, pH optimum, specificity of both the phosphate donor and the phosphate acceptor substrates, and molecular weight. The enzyme exhibits a broad specificity for the phosphate donor substrate; ATP and dATP are the most active, and GTP and dGTP the least active, but all nucleoside triphosphates tested, were able to serve as donors with the exception of the triphosphate end-products CTP and UTP, both of which are effective feedback inhibitors. In striking contrast, dCTP and dUTP are good phosphate donors. Sensitivity of the enzyme to the feedback inhibition is fully retained during the purification. Specificity of the purified enzyme for the phosphate acceptor substrate is much higher; in addition to the substrates uridine and cytidine, only 5-fluorouridine and the 5-aza- and 6-aza-analogs of uridine and cytidine are phosphorylated by the enzyme; thymine riboside, the deoxyribonucleosides, and cytosine arabinoside are not phosphorylated. The substrate specificity experiments are now being extended to explore the phosphorylation of 3-deazauridine and of pyrazomycin by this enzyme; both of these compounds have attracted recent clinical interest as anticancer agents and both presumably function as phosphorylated derivatives. The purified enzyme has a molecular weight of at least 150,000 and is presently being tested with Sephadex G-200 for a more definitive figure. When assayed at 37° C, the activity of the enzyme for uridine was approximately twice that for cytidine, and this ratio remained essentially constant throughout the purification. Initial velocity studies have been carried out with the purified enzyme, using a spectrophotometric assay that follows the reaction continuously. With either uridine or cytidine as phosphate acceptor, and ATP as donor, the initial velocity pattern is one of intersecting lines, rather than parallel lines, indicating that the reaction proceeds by the sequential addition of both substrates to the enzyme to form a ternary complex, followed by the sequential release of the two products. A ping-pong mechanism, in which the product of the first half-reaction is released prior to the addition of the second substrate to the enzyme, is ruled out by this pattern. Analysis of the data by Cleland's Sequen computer program have

indicated a K_m of the enzyme for uridine of 1.5×10^{-4} M, for cytidine of 4.5×10^{-5} M, and for ATP, with either uridine or cytidine as phosphate acceptor, of 3.6×10^{-3} M or 2.1×10^{-3} M, respectively. The V_{max} was 1.83 μmoles phosphorylated/min/mg enzyme protein for the uridine kinase reaction and 0.91 μmole for the cytidine kinase reaction. Thus, although the rate of uridine phosphorylation was twice that for cytidine, the K_m of the enzyme for cytidine is only one-third that for uridine. It is also of interest that although the activity for uridine was twice that for cytidine when the enzyme was assayed at 37° with different buffers and pH's, a lower temperature altered this ratio of the two activities in favor of uridine. Kinetic studies and product inhibition studies are now in progress to explore the nature of the competition between these two alternate substrates and to indicate whether both are phosphorylated at the same catalytic site on the enzyme or whether the two phosphorylations are mediated by separate sites or isozymes. The low affinity of the enzyme for ATP is also of interest, since the enzymatic activity is high primarily in tumor tissues, which have a high anaerobic metabolism and ATP concentration; the enzyme may require such conditions for high levels of activity. Further studies are also now in progress to elucidate details of the feedback regulation of the enzyme. With the purified enzyme, CTP appears to be twice as effective as UTP at high inhibitor concentrations, but both inhibitors are equally effective at the lowest inhibitor concentrations. The inhibition by CTP appears to be competitive with ATP, based on Lineweaver-Burk plots; however, a replot of the slopes against the CTP concentration is parabolic, and a Dixon plot is likewise parabolic. This indicates that the mechanism of inhibition by CTP is much more complex than one of simple, linear, competitive inhibition. Also, experiments suggest that the inhibition is non-competitive with the phosphate acceptor uridine and the Lineweaver-Burk plots appear hyperbolic, suggesting again a complex inhibition. However, no evidence of sigmoidicity has been observed in the substrate-saturation curve, and efforts to desensitize the enzyme to feedback regulation without destroying the catalytic activity have so far been unsuccessful. Possible allosteric qualities of the enzyme and its feedback regulation are therefore still an open question and are continuing to be explored. These experiments have been carried out by Mr. Alec Liacouras and myself. Two papers are in press on our purification and characterization of the enzyme and on our initial velocity studies that have defined the reaction mechanism. Mr. Liacouras has also completed writing his Ph.D. thesis covering part of these studies, and he will be finishing the requirements for the Ph.D. degree in Biochemistry at George Washington University in June, 1975.

In P815/FUR, a 5-fluorouridine-resistant subline of this tumor, we have shown that resistance to the analog is associated with a decreased level of uridine-cytidine kinase activity (Anderson, Cancer Res. 23: 1270, 1963; activity in crude extracts is as low as 10-20% of that found in extracts from the parent sensitive line). In order to compare the kinase from the resistant subline with that from the sensitive line, the enzyme has been purified 20-30 fold from the resistant cells, using methods similar to those developed for the purification from the sensitive tumor. The procedures had to be adjusted to the greater lability of the enzyme form from the resistant cells; this enzyme form, for example, requires higher ionic strength for the protection of activity. Although the resistant-line enzyme is also a high-molecular-weight protein, there is some evidence that it can be dissociated into enzyme forms that differ in molecular size; this heterogeneity could be related to the greater lability. A comparative study of the kinases from the two tumor lines showed that their kinetic parameters appear to be identical, as does the mechanism of the reaction. Thus, the initial velocity pattern again indicates a sequential addition of substrates to the enzyme to form a ternary complex. The K_m of the resistant-line enzyme for uridine was 1×10^{-4} M and for ATP 3×10^{-3} M. However, the resistant-line enzyme utilizes both dGTP and dATP as phosphate donors as efficiently as ATP, and the role of the triphosphate as an activator for the enzymatic reaction may be different for the two enzymes. Feedback inhibition by CTP and UTP is similar for both enzymes. Both the sensitive-line and resistant-line enzymes can use 5-fluorouridine as substrate, apparently to a comparable extent, so an altered substrate specificity does not appear to explain the resistance to the analog nor to account for the lower enzymatic activity in the resistant cells. These studies have been carried out largely by Dr. Jane M. Glick during postdoctoral fellowship in this laboratory.

2) Studies on UMP-CMP kinase. Our STRIDE trainee, Mr. Harry Free, in addition to helping with various research activities in progress, has also been engaged in a research project of his own to follow the levels of UMP-CMP kinase (reaction d above) and of uridine-cytidine kinase (reaction b) activities at different stages of cell growth. He has measured these kinase activities in developing cultures of Tetrahymena pyriformis to determine how the enzymatic activities increase during growth. We have previously purified UMP-CMP kinase 300-fold from Tetrahymena (Ruffner and Anderson, J. Biol. Chem. 244: 5994, 1969) and defined its reaction mechanism (Garvey, Millar, and Anderson, Biochim. Biophys. Acta 302: 38, 1973); Tetrahymena is a rapidly growing protozoan that is unable to synthesize pyrimidine or purine rings de novo and hence must depend on anabolism from the preformed ring structures, as in the scheme above. Since UMP is the central compound in pyrimidine nucleotide metabolism, the anabolism of this compound into the triphosphate precursors of nucleic acids should be related to the rapid synthesis of nucleic acids during active cell growth. Mr. Free's initial study assayed UMP kinase in cell lysates at different stages of culture growth, using a radioactivity assay employed in other experiments in the laboratory. Cell growth was measured both by total protein and by cell count, counting acid-fixed cells in a hemocytometer; this latter aspect was particularly challenging for him and required considerable research for an appropriate method, since live Tetrahymena are transparent to light and highly motile, rendering many usual counting methods useless. The increase in UMP kinase activity closely followed the increase in total protein and in cell count and did not precede the peak of cell growth. After growth leveled off, aging cells did not show an immediate decrease in UMP kinase activity, even though total protein did decrease. The kinase activity must therefore be functional in the maintenance of cell viability, probably in RNA synthesis,

and is hence required both for the increased production of RNA and DNA during growth and for the RNA activity throughout cell life. Comparable studies on the increases of uridine-cytidine kinase during growth are now in progress, and publication on both of these studies is anticipated in the near future.

3) Related activities. In addition to the biochemical studies, the work on uridine-cytidine kinase has made use, for the initial velocity and inhibition studies, of the DCRT central computer facility for routine calculations and for the more complicated problems of curve fitting, statistical evaluation of the data, and calculation of the K_m's, V_{max}'s, and rate constants for the reactions. In these computer operations, we have had a fruitful collaboration with Dr. F. K. Millar, Laboratory of Theoretical Biology, NCI.

Significance for Cancer Research and the Program of the Institute: UMP is the central compound in pyrimidine nucleotide biosynthesis in that it is the precursor for synthesis of other pyrimidine nucleotides of both the ribo- and deoxyribo- series. UMP synthesis by uridine kinase is called into play in rapidly growing tissues such as tumor tissues, and this constitutes a difference between normal and cancer cells. It is important to discover how the enzymes for UMP synthesis and metabolism are regulated in both types of cells, since such regulation is crucial in maintaining a balance among the levels of the various nucleotide precursors of nucleic acids and functions in regulating the rate of cellular nucleic acid synthesis. (NCP Objective 3, Approach 2).

Proposed Course of Research: Further studies on the purified uridine-cytidine kinase from the P815 tumor will use kinetic competitions and product inhibitions with the aim of answering whether the two normal substrates uridine and cytidine, and the analog substrate 5-fluorouridine, compete for a single, identical catalytic site on the enzyme or whether they are, in fact, phosphorylated by separate sites or isozymes. The kinetic parameters of activity of the enzyme with the new analogs 3-deazauridine and pyrazomycin will also be quantitatively defined. Additional studies will also explore further the way in which the feedback inhibitions by CTP and UTP regulate the phosphorylation of the respective nucleoside substrates cytidine and uridine and the way in which they compete with ATP. If ways can be found to stabilize the enzyme to further purification, studies on the mechanism of the feedback regulation will be extended to encompass explorations on the detailed molecular structure of the enzyme.

Mr. Free hopes to extend his study of anabolic enzymatic activities during cell growth to measurements of UMP pyrophosphorylase (reaction c) activity, since this is a functional and perhaps even crucial enzyme in this organism, in contrast to the situation in mammalian cells. Both this enzyme and the uridine-cytidine kinase (reaction b) precede UMP-CMP kinase (reaction d) in the anabolic pathway in *Tetrahymena* and either might therefore be rate-limiting in the increase in the pathway during cell growth.

With Mr. Free's recently completed training under the STRIDE program and his increased professionalism, this study also promises to be fruitful and to yield fully publishable results.

Keyword Descriptors:

Nucleic acid synthesis, pyrimidine metabolism (uridine, cytidine, UMP, CMP), feedback regulation, resistance to antimetabolites, kinases, enzymatic reaction mechanisms, kinetics, initial velocity studies, tumor cell lines

Publications:

Liacouras, A. S. and Anderson, E. P.: Uridine-Cytidine Kinase I. Purification from a Murine Neoplasm and Characterization of the Enzyme. Archives Biochem. Biophys. 168, May, 1975 (in press)

Liacouras, A. S., Garvey, T. Q., III, Millar, F. K., and Anderson, E. P.: Uridine-Cytidine Kinase II. Kinetic Studies and Reaction Mechanism. Archives Biochem. Biophys. 168, May, 1975 (in press)

Liacouras, A. S.: Purification of Uridine-Cytidine Kinase from a Murine Neoplasm and Characterization of the Enzyme with Regard to Properties and Reaction Mechanism. Ph.D. Thesis in Biochemistry, George Washington University, 1975.

Project No. Z01 CBD 00306-12 LPHY
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of β-hydroxy-α-amino acids

Previous Serial Number: Same

Principal Investigator: Theodore T. Otani

Man Years:

Total:	2
Professional:	1
Others:	1

Project Description:

Objectives: To prepare chemical compounds which preferentially destroy cancer cells by mechanisms other than those of agents presently being used in cancer chemotherapy. Earlier experiments have shown that some of the β-hydroxyamino acids prepared in this Laboratory exhibited growth-inhibitory activity in microbial systems selected for anti-tumor screening, and probable tumor-inhibitory action in some mammalian systems. Preparation of these compounds and separation of their isomers have been undertaken in anticipation of more extensive anti-tumor studies in mammalian systems.

More specifically, the immediate objectives of this portion of the project have been:

(a) To prepare β-hydroxynorleucine, which have been shown previously to have possible anti-tumor activity in microbial anti-tumor screening system, to isolate its four isomers, and to prepare the N-chloroacetyl derivatives of these isomers.

(b) To prepare other related β-hydroxyamino acids, such as the methionine derivatives, β-hydroxymethionine, β-hydroxyhomomethionine, β-hydroxymethoxinine, chain-substituted halo-aliphatic amino acids, such as: γ-dichlorothreonine, γ-trifluorothreonine, and γ-chloro-β-hydroxynorleucine.

(c) To isolate the four isomers of each of these compounds and to prepare the N-acyl derivatives of each isomer.

(d) To prepare the N-acyl derivatives of other amino acid analogs which have been shown to possess antimetabolic properties.

(e) To test these compounds, both the free amino acids and the N-acyl derivatives, for anti-tumor activity, first in microbial screening system, and then, if found active, and when obtained in sufficient quantities, in mammalian system.

Methods Employed: The β-hydroxyamino acids are prepared by methods developed in this Laboratory. An appropriate aldehyde is condensed with glycine in the presence of catalytic amounts of cupric ions (or with glycine-copper complex) in basic medium under conditions which are empirically determined for each compound.

Major Findings:

(1) The four isomers of N-chloroacetyl-β-hydroxynorleucine have been prepared in quantities minimally sufficient for testing in three mammalian tumor systems. The purity of these compounds was ascertained by a) optical rotation determination, b) elemental analysis, c) melting point determination, d) Van Slyke nitrous acid determination of primary amino nitrogen, and e) chromatography of the non-acylated mother compounds in five solvent systems.

(2) Test for possible anti-tumor action using microbial screening system indicated that although the non-acylated mother compounds of these isomers were not growth-inhibitory, the N-chloroacetylated derivatives of all four isomers showed modest but significant growth-inhibitory activity. The N-chloroacetyl-D-enantiomorph of diastereomer B exhibited the greatest activity, showing about twice that of the other three isomers.

(3) The observation that the acyl derivative of both optical isomers were active indicated the improbability of the involvement of an acylase in the mechanism of inhibition, since the D-antipodes of the chloroacetyl derivatives of neither diastereomer were substrate of that enzyme.

(4) In view of the above findings, N-acyl derivatives of other amino acid analogs have been prepared. Chloroacetyl-DL-allylglycine, chloroacetyl-DL-β-2-thienylalanine, and chloroacetyl-DL-p-fluorophenylalanine have thus far been prepared.

(5) Preliminary studies in the preparation of a new β-hydroxyamino acid, i.e., β-hydroxytryptophane (by alkaline condensation of indole-3-carboxyaldehyde with glycine-copper complex) have been made, and formation of modest amounts of the product have been observed on paper chromatogram.

Significance for Cancer Research and the Program of the Institute: That amino acid metabolism may be a susceptible area of action in cancer therapy was demonstrated by the efficacy of asparaginase in certain tumors which required asparagine. Amino acid deprivation, either by the removal of the amino acid or by the substitution with an analog may be effective in inhibiting tumor growth.

The unnatural amino acids synthesized under this program are close structural analogs of essential natural amino acids, differing in some cases only in the presence of a single hydroxyl group adjacent to the α-amino group. Hence, β-hydroxynorleucine differs from norleucine only in the presence of a hydroxyl group in the β-position of carbon chain of the amino acid. β-Hydroxynorleucine is a structural analog of methionine, differing from the latter in having a methylene group in place of the sulfur atom in the γ-position of the chain. Indeed, β-hydroxynorleucine, even as a mixture of four isomers, not only exhibited inhibitory action in two microbial systems, but also showed some anti-tumor effect against Carcinoma 755 as measured by the decrease of tumor size and increase in survival time. β-Hydroxymethoxinine and β-hydroxymethionine are even closer analogs of methionine than is β-hydroxyhomomethionine, the former differing from methionine in possessing an oxygen atom in place of the S atom and in possessing a β-hydroxyl group, and the latter only in possessing a β-hydroxyl group.

The chain-halogenated amino acids, similarly, are close structural analogs of essential amino acids. For example, γ-dichlorothreonine would be an analog of leucine, since the size of the chlorine atom is approximately that of the methyl group; also γ-triflurothreonine would be a close analog of threonine since the atomic radius of the fluorine atom is essentially that of hydrogen atoms. Objective 6, Approach 2.

Proposed Course of Research:

(1) To test the four isomers of N-chloroacetyl-β-hydroxynorleucine for anti-tumor activity in mammalian systems.

(2) To prepare the optically pure isomers of other amino acid analogs known to be anti-metabolites, and to prepare the N-acyl derivatives of these compounds.

(3) To test each of these optically pure N-acyl amino acid analogs for anti-tumor activity, first using microbiological anti-tumor screening system (which require very small quantities of the test compounds), and then, on mammalian tumor systems.

(4) To continue the attempts to isolate the four isomers of β-hydroxymethoxinine and of β-hydroxy-homomethionine.

(5) Prepare γ-halo-substituted β-hydroxyamino acids, which are possible anti-metabolites of threonine.

Keyword Descriptors:

β-Hydroxyamino acids, β-hydroxynorleucine, chloroacetyl-β-hydroxynorleucine, diastereomers

Publications:

Otani, T. T. and Briley, M. R.: β-Hydroxyhomomethionine and β-hydroxymethoxinine: Preparation and Separation of Diastereomers. J. Pharm. Sci. 63: 1253-1256, 1974.

Project No. Z01 CBD 00944(a)-13 LPHY
1. Laboratory of Physiology
2. Energy Metabolism Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Total Metabolism

Previous Serial Number: Same

Principal Investigator: Seoras D. Morrison

Man Years:

Total:	2.5
Professional:	1.0
Others:	1.5

Project Description:

Objectives:

(a) Investigation of the patterns of heat production of rats with concomitant recording of feeding behavior and other activity with a view to finding how the energy expenditure, feeding and general behavior pattern are related to tumor growth and to finding the nature of the loads imposed by a tumor on the host.

(b) Investigation of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth.

(c) Examination of possible experimental methods of simulating in non-tumor-bearing rats the cachexia and decline of food intake found during neoplastic growth.

(d) Development of quantitative and reproducible assessment of behavioral output, to yield more sensitive and earlier detection of systemic effects of tumors.

Methods Employed: The methods of indirect calorimetry, operant conditional responses, placement of electrolytic lesions in or stimulation of the central nervous system, continuous or programmed infusions into unrestrained animals, and methods of orthodox nutritional studies. Ballistic methods of recording and partitioning total motor activity. Computer methods of numerical analysis of serial records of tumor growth and of continuous records of gaseous exchange. Sprague-Dawley rats and Buffalo rats are used in all animal experiments. Most tumor-bearing rats are inoculated with Walker 256 carcinoma, but other transplantable tumors are also used.

Major Findings:

(a and b) Change in diurnal variation of feeding and motor activity in cachectic tumor-bearers is similar to change in diurnal variation induced by chronic hypothalamic damage (Cancer Res. 34, 1632-1635). This is the first evidence of a connection between the cachectic process and dysfunction of central control of feeding.

(c) Feeding responses to nutrient density, environmental temperature and exogenous insulin are distorted by tumor growth. The precise patterns of distortion are complex and are being investigated. In general, feeding responses of tumor-bearers to feeding stimuli are attenuated but there appears to be substantial dependence on the effectiveness of the pre-cancerous control of feeding of the host.

(d) Changes in feeding pattern in tumor growth and the extent to which food intake is modified by change in amount of feeding activity or change in feeding efficiency are being examined with respect to various normal influences on food intake and to changes with tumor growth and brain damage, separately and in combination. Computer analysis of long-period records of meal patterns is being developed in collaboration with Dr. F. K. Millar (Laboratory of Theoretical Biology, DCBD).

Significance for Cancer Research and the Program of the Institute: The findings on control of feeding in tumor growth are throwing light on the nature of cancer cachexia and should be utilizable in the development of effective methods for improving the nutritional condition of cancer patients. The section of the National Cancer Plan that the work most closely approximates is: Objective 6 (Develop the means to cure cancers and to retard the progress of cancers not cured). Approach 4 (Enhance the host's ability to eliminate or prevent further development of cancer). It is also immediately relevant to the 1974 Amendment to the Cancer Act (Collect ..... information respecting nutrition programs for cancer patients and the relationship between nutrition and cancer).

Proposed Course of Project: Work on energy and water exchange of tumor bearers will be continued along with study on the interaction of metabolic and behavioral responses of normal and cancerous animals, with particular reference to the reasons for the changes in food and water intake and depletion of host tissues that occur during tumor growth. Objective 6, Approach 4.

Keyword Descriptors:

Project Title: Cancer cachexia, nutrition, food intake, energy exchange, water exchange

Project Description: Cancer cachexia, nutrition, food intake, control of feeding, energy exchange, water exchange, feeding behavior, calorimetry, central nervous surgery, data acquisition, data processing

Publications:

Morrison, S. D.: Sodium and the cachexia and hypophagia of tumor growth. J. Natl. Cancer Inst. 52: 869-874, 1974.

Morrison, S. D.: Diurnal distribution of motor activity and feeding during growth of tumors. Cancer Res. 34: 1632-1635, 1974.

Morrison, S. D.: Energy Balance. Nutr. Rev. 32: 191, 1974.

Project No. Z01 CBD 00919-12 LPHY
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Urinary Excretion Patterns in Leukemia

Previous Serial Number: Same

Principal Investigator: James C. Reid

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: This project is a developmental effort to broaden the scope of urinary analysis. Its objectives are the development of methodology for estimating simultaneously the concentrations of many components of a urine specimen and the evaluation of such information for potential utility from both the clinical and biological standpoints. Interest has focused especially on nucleic acid congeners but is not confined thereto.

Methodology: The basic concept has been that it may be feasible to devise an analytical system powerful enough to deal with the complexity of urinary composition by the application of computational technique for multicomponent mixture analysis to urinary fractions obtained after preliminary chromatographic simplification. The use of ultraviolet spectra as input data for such technique has been evaluated and found to be infeasible as detailed earlier. Alternate approaches are under consideration.

Major Findings: A final review of the extensive data obtained in earlier work fails to yield any conclusions which could be considered reliable enough to merit further work on multicomponent urinary analysis in the cancer context, at least as far as small molecules are concerned. A somewhat similar program conducted at Oak Ridge appears to have had a comparable outcome (J. Natl. Cancer Inst. 53: 1553-1563, 1974). The essential problem is inability to determine the concentrations of individual components of chromatographic fractions, which is essential because the chromatographic profiles do not exhibit sharply differentiated patterns.

Since small-molecule analysis appears infeasible as a basis for cancer diagnosis, attention turns to the macromolecules such as nucleic acids which might yield useful information embodied in nucleotide sequences. Methodological difficulties would arise from the difficulty of assaying the very small amounts of such substances which occur in urine, but might be overcome by the application of replication procedures in vitro to amplify the quantity available for assay by a variety of sensitive techniques which are available. Currently the literature is being reviewed to estimate the feasibility of such an approach,

Significance for Cancer Research and the Program of the Institute: The basic concept guiding this project is that the most clearly defined potential for achieving a near-term improvement in cancer morbidity data resides in the development of a diagnostic procedure which would reveal the presence of cancer early enough to permit intervention before metastasis has begun. National Cancer Plan, Objective 5 (diagnosis), Approach 4.

Proposed Course of Research: Undetermined, depending on the outcome of a literature survey now in progress.

Keyword Descriptors:

Urinary, patterns, multicomponent, leukemia

Project No. Z01 CBD 00936-05 LPHY
1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Recovery from effects of radiation or other initial treatment in terms of tolerance to subsequent treatments

Previous Serial Number: Same

Principal Investigator: Willie W. Smith

Man Years:

Total:	5
Professional:	1
Others:	4

Project Description:

Objectives: To study the course of recovery from irradiation of the whole body or of the leg-shielded animal in terms of ability to survive an additional exposure.

Major Findings: It has been shown that whole-body irradiation in mice leaves a small amount of irrepairable damage which reduces tolerance to a subsequent exposure. One object of the present study is to identify the site and nature of this damage. In leg-shielded animals we see no evidence of such a residual damage. It therefore appears that this damage is associated with the bone marrow rather than the gastrointestinal tract.

Significance for Cancer Research and the Program of the Institute: There is increasing interest in therapeutic efficacy of combined treatment modalities, especially including radiation and chemotherapy. We have made several studies of this nature but digressed in order to gain a better understanding of the effects of repeated irradiation as specifically related to hematopoietic and gastrointestinal damage and recovery. Objective 6, Approach 1.

Proposed Course of Project: The studies are being extended to include relationships between dose and residual damage in survivors of higher initial radiation in shielded and unshielded mice. With this background we hope to resume studies of combined radiation and chemotherapy.

Keyword Descriptors:

Persistence of radiation effects, irrepairable radiation damage

Project No. Z01 CBD 00941-18 LPHY
1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunogenetic factors affecting bone marrow transplantation in irradiated mice.

Previous Serial Number: Same

Principal Investigator: Delta E. Uphoff

Man Years:

Total:	3.5
Professional:	1.0
Others:	2.5

Project Description:

Objectives: To facilitate bone marrow transplantation as an adjunct to administering toxic levels of either irradiation or chemotherapeutic drugs in the treatment of cancer.

Methods Employed: Inbred mice, reciprocal F_1 hybrids and especially derived substrains are exposed to lethal doses of X-irradiation and engrafted with bone marrow cells from different donors to test the effect of differing degrees of genetic disparity on survival. Attention is focused primarily on the production and prevention of the graft-versus-host (GVH) reaction as a primary obstacle for clinical marrow transplantation.

Major Findings: The investigation of male specific histocompatibility (H-Y) antigens was continued using marrow transplantation in reciprocal hybrid mice. Completed data is available for one pair of hybrids in which the parental strains represent a major (H-2) antigenic difference. The genetically identical reciprocal hybrid females differ in their ability to respond to the same H-Y antigen as a result of a maternal influence on responsiveness. Each hybrid female also produced a characteristically different response to the H-Y antigens of reciprocal hybrid males. However, both females produced the strongest response to the same H-Y antigen, indicating that the H-Y antigens form an allelic series rather than a modified expression of the same antigen as in the case of a maternal influence. The critical factors for survival are the relationship between responsiveness of females and antigenicity of H-Y antigens for these factors establish the severity of the GVH reaction. A GVH reaction may also occur when male marrow is grafted to female recipients as a result of an X chromosome disparity. The extent to which the X chromosome affects survival is being investigated.

The investigation of the maternal influence on antigenicity and responsiveness was continued. This project is of primary importance because the NIH colony of inbred mice has been foster nursed during its establishment as a specific pathogen-free colony. All mice obtained from this facility are now products of ancestors nursed by foster mothers of unknown genotypes and may exhibit maternal influences in their ability to recognize and respond to certain antigens. An additive effect of maternal influences on two substrains of C3H foster nursed on inbred females of different H-2 genotypes was demonstrated. The ancestors of C3HfB were foster nursed on a C57BL($H-2^b$) mother and those of C3HfD were foster nursed on an NBL($H-2^d$) mother. Reciprocal marrow transplantation experiments produced vastly different GVH reactions lethal to 18% of the C3HfD recipients of C3HfB marrow and 72% of the C3HfB recipients of C3HfD marrow. NBL mice consistently produce a more severe response than C57BL/He mice and this difference in responsiveness was transferred to the foster-nursed substrain. Although these genetically identical mice were not obtained from the NIH colony, they clearly demonstrate the effect this simple procedure of foster nursing may have on experimental results. Consequently, the NIH colony may no longer be considered "type strain" of mice.

The investigation of the hybrid-versus-parental strain reaction involving the recognition of quantitative differences in antigenicity was continued and expanded to analyze possible X and Y chromosome effects.

Significance for Cancer Research and the Program of the Institute: Bone marrow transplantation has furnished a very sensitive method of studying the antigen recognition system, antigenic expression, degrees of responsiveness and maternal influences. As reciprocal hybrids become accepted as a requirement for immunologic investigations, basic concepts will undoubtedly need revision. Since degrees of responsiveness and qualitative and quantitative differences in antigenicity are detectable in *in vivo* systems, it is necessary to refine the available *in vitro* test or develop new tests capable of detecting these subtle differences recognized by the intact immune system. This information is applicable to immunotherapy of tissues and clinical marrow transplantation. Objective 6, Approach 1.

Proposed Course of Project: No changes are anticipated. Greater emphasis will be placed on the investigation of the response of F_1 hybrids to parental strains as experiments involving the X and Y antigens are concluded. The H-2 specificity of maternal modifications will be investigated as time and space permit. Other aspects of this project reported in previous years are being continued according to the most efficient utilization of the available mouse strains.

Keyword Descriptors:

Bone marrow transplantation, irradiated mice, graft-versus-host reaction, maternal influences, histocompatibility, immunogenetics, phenotypic modifications

Publications:

Uphoff, D. E.: Maternal influence on responsiveness of multiparous mice to antigenic characteristics of their male progeny. J. Natl. Cancer Inst. 52: 1843-1847, 1974.

Jacobs, B. B., and Uphoff, D. E.: Immunologic modification: A basic survival mechanism. Science 185: 582-587, 1974.

Uphoff, D. E.: Comparative survival of lethally irradiated inbred male mice inoculated with marrow from virgin or multiparous female donors. J. Natl. Cancer Inst. (in press)

Uphoff, D. E.: Maternal influences: Their immunologic aspects. In Mühlbock, O. (Ed.): Basic Aspects of Freeze Preservation of Mouse Strains. UNESCO (in press)

Project No. Z01 CBD 00942-16 LPHY
1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of Ionizing Radiation on Nucleic Acids, Proteins and Their Constituents: Radioprotective and Radiosensitizing Agents

Previous Serial Number: Same

Principal Investigator: Peter Riesz

Other Investigators:
S. Kominami, Visiting Fellow
V. Wee, Visiting Fellow

Man Years:

Total: 5.5
Professional: 3.0
Others: 2.5

Project Description:

Objectives: The effects of ionizing and ultraviolet radiation on biological macromolecules and their constituents are being investigated. For reproductive death, DNA is the target molecule in viruses and is at least a part of the target molecule in bacteria and mammalian cells. Radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals or by the "indirect effect" where radical species formed in the surrounding medium by radiation react with DNA. In the case of water, these species are hydrated electrons, hydrogen atoms and hydroxyl radicals. In the case of radiation damage to the chromosome, radicals formed in the nucleohistone may cause damage to the DNA.

In the chain of events that leads to loss of biological activity, free radicals play an important role. We have found recently that the addition of certain metal ions to DNA in the ratio of 1 metal ion per 10 nucleotides, can reduce the radical yield by a factor of about 10.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity of these macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

Methods Employed: When proteins or nucleic acids are irradiated with gamma rays or ultraviolet radiation in the absence of air (at 10^{-4} pressure), trapped free radicals are generated.

Trapped radicals in proteins and nucleic acids were produced by:

(a) Gamma-radiolysis using a Co^{60} source.

(b) Photolysis with 2537 Å light using a low pressure Mercury arc.

Electron Spin Resonance studies were carried out using a Varian E-9 ESR Spectrometer.

Proteins and DNA were tritium labeled on exchangeable sites by exposing the dried materials to tritiated water vapor (5 curie/gm) at 20 torr for 10 minutes in a high vacuum system located in a hood in the Isotope Laboratory (Building 21).

Major Findings:

1. The Effect of Visible Light on γ-Irradiated DNA and its Constituents at 77° K. (with S. Kominami, V. Wee and H. Moss)

The effect of visible light on DNA in the solid state, previously γ-irradiated in the dark at 77° K in vacuo or in a helium atmosphere, was investigated by electron spin resonance. The ESR signal at 77° K decreased by about 75% after illumination with visible light at 77° K. The remaining radical population did not decrease when the temperature was raised from 77° K to 298° K. The same thermal annealing process applied to non-illuminated DNA decreased the radical content by about 75%. From an investigation of the effect of thermal annealing at various temperatures in the 77° K to 300° K range followed by illumination with visible light, the remaining radical population in all cases consisted of the 5,6 dihydro-5-thymyl radical (with a characteristic octet of ESR lines) plus an unidentified species which has a singlet absorption. This population was stable to light but could be transformed by heating in the 380° K - 410° K range into a new radical species without loss of radical content.

For adenine, cytosine and guanine, seventy-five percent or more of the radicals at 77° K could be removed by visible light (for thymine and deoxyribose about 20%); while for the corresponding nucleosides the percentage of light-sensitive radicals was considerably smaller. The radical content of thymidine at 77° K, a substance for which previous single crystal studies at 77° K indicated only neutral radicals, remained unchanged by illumination. The number of radicals in illuminated bases and nucleosides decreased much less on warming from 77° K to 298° K than in non-illuminated samples; however, the overall decrease in radical content for illumination at 77° K followed by warming to 298° K was approximately equal to that obtained for heat treatment alone. These results suggest that the ionic radicals produced by γ-radiolysis at 77° K in DNA and its constituents are light sensitive.

2. Effects of Metal Ions on the Formation of Strand Breaks and Radicals and on Hydrogen Transfer in γ-Irradiated DNA (with S. Kominami, V. Wee and H. Moss)

The effects of various metal ions (Cd^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cr^{3+}), present at a concentration of 1 metal ion per 100 nucleotides, on the formation of carbon radicals at 77° K and at 298° K, on the production of phosphomonoester groups, and on hydrogen transfer in γ-irradiated DNA in the solid state in vacuo were investigated. Those metal ions which decrease the radical yield at 77° K and 298° K (as measured by electron spin resonance) were also effective in decreasing the formation of strand breaks and the transfer of hydrogen from exchangeable to carbon-bound sites. These observations can be explained by assuming that the metal ions decrease the yield of a common precursor species.

From ESR studies of cupric-DNA complexes, it was inferred that cupric ions compete with the bases in DNA for electrons produced by γ-radiation, thus preventing the formation of anionic radicals at 77° K which are precursors of the stable neutral radicals observed at room temperature.

3. The Effect of Oxygen on γ-Radiation Induced Strand Breaks in DNA (with V. Wee and H. Moss)

The G value (the number of molecules produced per 100 ev absorbed) for monophosphoester formation in γ-irradiated DNA in the solid state was found to be 1.5 in the absence of air. When γ-radiolysis of DNA is carried out in the presence of oxygen, the G value is increased to 16.0. Such a large G-value implies a chain reaction in the solid state involving the deoxyribose part of the molecule. Irradiation of DNA in vacuo followed by dissolution in aerated or deaerated water produces monophosphoester with a G value of 1.5. Hence, the chain breaks result from pre-existing breaks in the solid prior to dissolving in water.

γ-Radiolysis of DNA at 77° K in vacuo, followed by exposure to liquid oxygen at 77° K and subsequent warm-up to room temperature produced a much smaller yield of strand breaks than γ-radiolysis in the presence of oxygen at room temperature.

The large protective effect of metal ions in preventing the formation of strand breaks in DNA irradiated in the absence of air, is completely suppressed by oxygen. Oxygen appears to act both as an electron and as a radical scavenger at an early state of the radiation damage to bring about a chain reaction in the solid state with an approximate 10-fold increase in strand breaks. This result is of particular interest in connection with the recent observation by K. C. Smith and co-workers of a 10-fold increase of strand breaks due to oxygen in irradiated mammalian cells under conditions where all repair processes are minimized. Hence, it follows that the "direct effect" of radiation on DNA shows the same oxygen effect as that observed in mammalian cells under conditions of minimal repair.

4. X-Ray Analysis and ESR Studies of Copper-Doped Crystals of Cystine Dihydrochloride Dihydrate (with S. Kominami; J. Silverton and T. Akiyama, LC, NHLI)

A new crystalline form of cystine dihydrochloride containing 2 water molecules per cystine was found. These crystals were obtained only by slow crystallization from deaerated hydrochloric acid solutions containing a high concentration of cupric ions. The crystals used for X-ray diffraction and ESR analysis contained about 0.01% of cupric ions. The crystals of the copper-doped L-cystine hydrochloride dihydrate are monoclinic with space group $P2_1$, while those of cystine hydrochloride are monoclinic with space group C2, and contain no water. The positions of all of the atoms in the unit cell were determined by X-ray diffraction and the structure was refined to a final r-value of 0.051.

A deuterated copper-doped crystal of cystine dihydrochloride dihydrate was also obtained. The ESR spectra were measured at 77° K by rotating these crystals in the static magnetic field around the crystallographic axes at 5° intervals. Two different complexation sites for cupric ions could be distinguished. The first one was in a square planar ligand field with water and chloride ion making one side of the square. The third ligand was the oxygen of a carboxyl group and the fourth (which required some distortion of the crystal structure) was either a carboxyl oxygen or a sulfur of the disulfide group. The second compluxation site of copper is also square planar with 2 amino groups and 2 chloride ions of ligands and requires no distortion.

Significance for Cancer Research and the Program of the Institute: Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy, (2) carcinogenesis, (3) stability of the genetic pool, (4) the suppression of the immune mechanism, and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained. Objective 6, Approach 1.

Proposed Course of Project: To continue studies on the effects of ionizing radiation on macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents will be investigated.

Keyword Descriptors:

Gamma radiation, DNA, free radicals, electron spin resonance, radioprotective and radiosensitizing agents

Honors:

Completed a 3-year term on the Editorial Board of Radiation Research

Chairman, Session on Biochemical Changes Induced by Radiation: DNA and Proteins. Fifth International Congress of Radiation Research, Seattle, July 1974

Publications:

Kominami, S. and Riesz, P.: Effects of metal ions on the γ-radiolysis polycrystalline djenkolic acid. Radiat. Res. 58: 154-165, 1974.

Friedberg, F., Kominami, S., Nandedkar, A. K. N. and Riesz, P.: Effects of metal ions on the formation of chain breaks and radicals in γ-irradiated collagen and polyadenylic acid. Radiat Res. 61: 55-62, 1975.

Kominami, S., Wee, V. T., and Riesz, P.: Effects of metal ions on the formation of radicals and strand breaks and on hydrogen transfer in γ-irradiated DNA. Radiat. Res. (in press).

LABORATORY OF PATHOPHYSIOLOGY
SUMMARY STATEMENT
ANNUAL REPORT
July 1, 1974 through June 30, 1975

The primary objective of the Laboratory of Pathophysiology is the understanding of conditions which determine tumor regression or initiation, regulation and arrest of growth of neoplastic tissues. Seventeen projects are presently carried on with this major objective in mind.

Six projects deal with the mechanism of mammary tumor regression secondary to hormonal deprivation or cyclic AMP treatment of the host. Previous work, carried on in this laboratory, convinced us that a high level of lysosomal activity is not the primary cause of tumor destruction, as it occurs after hormone withdrawal, but a secondary event, or "cleaning up" type of operation. This conclusion is based on three observations: (1) increase of lysosomal enzyme activity in some regressing tumors occurs when regression is well advanced; (2) arrest or severe impairment of protein and RNA synthesis does not alter the time sequence of regression; and (3) the lysosomal enzymes remain localized within the cells and do not spill over into the interstitial compartment. It seems very unlikely that cell membranes would be spared if lysosomes were the primary agents of cell digestion, while it would be consistent with the normal behavior of lysosomes to remain within the cell to digest unneeded or altered materials. Several approaches are being followed in searching for events initiating cell autodigestion.

Dr. Cho-Chung is studying the role of cyclic AMP and has found that: (1) Injections of dibutyryl cAMP may indeed produce regression in some rat tumors. (2) Regression does not depend on the level of cAMP in the tissue but on the presence of the protein-binding capacity of the tumor cytosol. (3) From one line of transplanted W256 mammary carcinomas, two lines were separated *in vivo*, one growing and one regressing under dibutyryl cAMP treatment. A qualitative and quantitative difference in cytosol binding capacity was found in the two lines. (4) Prostaglandin E_1 treatment of tumor slices, from both responsive and unresponsive lines, produced a significant induction of basal and NaF-stimulated adenyl cyclase and cAMP-phosphodiesterase (the two enzyme systems which regulate cAMP level) only in the line regressing under dibutyryl cAMP treatment.

Since the cAMP binding protein is related to the regulatory subunit of protein kinase, Dr. Yi is studying the activation of protein kinase in the two lines of tumors, regressing and growing after dibutyryl cAMP treatment, respectively. Dr. Yi finds that cAMP dependent protein kinase activity is increased by prostaglandin E_1 treatment only in tumors regressing after cAMP treatment, not in the cAMP unresponsive tumors. Moreover, the catalytic subunit of protein kinase is similar in both types of tumors, but the regulatory subunit appears to be different, and this difference is related to cAMP-responsiveness.

The attempt to define the triggering mechanism of the regression process is also following another approach. Dr. Rouleau is systematically analyzing electrophoretic patterns of nuclear and cytoplasmic proteins for detectable differences; Dr. Gullino is following the same approach for RNA. No specific findings are available at this time. Dr. Bodwin has observed that the microsomal isozyme of glucose-6-phosphate dehydrogenase disappears in tumors regressing under cAMP treatment. Preliminary experiments indicate that modifications in the isozyme quantities are also occurring during regression after hormonal deprivation. Purification of G6PD isozymes is now going on with the objective of quantitating changes in the isozyme pattern and correlating these with tissue regression. Following the same kind of approach, Dr. Qasba has selected several mammary carcinomas, of rodent and human origin, which are able to synthesize α-lactalbumin. He is now studying both the protein and RNA message for it and looking for possible changes which may be related to the regression process. Finally, Dr. Knazek, who has developed a method for growing solid masses of tissue on artificial capillary networks, is trying to reproduce *in vitro* the hormone-dependent regression observed *in vivo*. This attempt is based on the need to reproduce the regression process in a more homogeneous cell population and reduce the complexity of the *in vivo* model system.

Five projects are concerned with events occurring in cells at rest or at the initiation of mitosis. Drs. Cooper and Berger are studying RNA in lymphocytes, resting or dividing, under stimulation by mitogens. They observed that: (1) The largest molecules of heterogeneous RNA are poorly represented in the pool of poly(A)-bearing heterogeneous RNA which turns over with a half life of 7 hours and maintain a rather constant average size during the processing period. (2) Resting lymphocytes contain about 70% of their cytoplasmic ribosomes as single 80S monomers. More than 80% of these monomers were found easily dissociable in high salt concentration, therefore are inactive (free ribosomes). (3) New rRNA first entered native 40S and 60S subunits and polysomes. As the particles aged, they accumulated in the pool of inactive free ribosomes but there was no rapid interchange between inactive free ribosomes and newer native subunits. (4) Following growth stimulation with phytohemagglutinin, protein synthesis increased and reached a peak at 14-16 hours; virtually no inactive ribosomes remained. Accumulation of inactive ribosomes started again 20 hours after stimulation. (5) Two classes of poly(A)-bearing RNA were found in resting lymphocytes with a half life of less than 17 minutes and more than 24 hours, respectively. In lymphocytes, dividing after mitogen stimulation, the half life of mRNA deviates markedly from that found in resting lymphocytes. The data from observations (1) to (5) are compatible with the following interpretation: The resting lymphocyte polyadenylates new heterogeneous RNA which survives longer. Whether this is part of the mechanism for the regulation of protein synthesis has yet to be clarified. In resting lymphocytes, protein synthesis is minimal, and most ribosomes are inactive until stimulation with a mitogen occurs. Rapid activation of ribosomes is probably due to the availability of a "dissociation" factor, while mRNA appears to be limiting at maximum levels of protein synthesis.

Dr. Kidwell is analyzing the sequence of events involved in the initiation of mitosis. He found that poly(ADP-Ribose) is being synthesized at the end of S-phase and beginning of G_2 phase. Substances, such as adriamycin, which arrest cell cycle progression also produce accumulation of cells with elevated poly(ADP-Ribose) polymerase activity, but this does not occur with substances, such as bichloronitrosourea, which act on the late G_2 phase. By the use of

5-methylnicotinamide, which inhibits poly(ADP-Ribose) synthesis, a variant of HeLa cells has been developed which continues to produce poly(ADP-Ribose) and also survives. The resistance to 5-methylnicotinamide of poly(ADP-Ribose) polymerase appears to be higher in the resistant cell population. Thus, a causal relationship between inhibition of cell division and inhibition of poly(ADP-Ribose) synthesis seems to have been demonstrated. The role of this compound in the arrest of tumor growth is now being studied.

Work on five more projects is going on in the area of general tumor pathophysiology. (1) Dr. Yi is studying temperature regulation by the solid tumor with the objective of gaining basic information badly needed to rationalize tumor treatment by heat. (2) Dr. Qasba is completing work on the ability of isolated nuclei of SV_{40}-infected cells to produce RNA and to degrade it according to the temperature of the assay mixture. (3-4) Dr. Knazek is expanding the range of possibilities of the capillary cell culture unit with the main purposes of determining if (3a) bone barrow of a donor can be kept viable and differentiated to replace the original marrow destroyed by radiation; and (4b) if human macromolecules, such as prolactin or growth hormone, can be produced by human cells maintained in the capillary culture unit. (5) Dr. Brem is using induction of neovascularization in the rabbit's eye as an identification of neoplastic transformation of human mammary gland at an early stage. The procedure was proven effective in our laboratory for mouse mammary gland by Dr. M. Gimbrone.

Project No. Z01 CBD 00368-05 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Mechanism of Mammary Tumor Regression.

Previous Serial No.: NCI 368 (and NCI 8202)

Principal Investigator: Pietro M. Gullino

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1 1/2
Professional: 1/2
Others: 1

Project Description:

Objective: To understand the mechanism of regression in hormone-responsive mammary tumors.

Methods Employed: Tumor regression is obtained by castration in primary tumors induced by 7,12-DMBA or N-nitrosomethylurea. In transplantable MTW9 regression is obtained by removal of the mammotropin source, usually MtTW10 pituitary tumor. Standard techniques were used to infuse radioactive isotopes through the aorta, separate tissue and cell fractions, and analyze RNA in sucrose gradients and acrylamide gels.

Major Findings: (1) Work done in the past convinced us that increased lysosomal activity during regression cannot be the cause of tumor destruction but is the consequence of alterations occurring within the cells which in turn stimulate lysosomal function. (2) In the search for the kind of alteration, if any, occurring in cells when hormone is withdrawn, we observed that: (a) Actinomycin D or cycloheximide can arrest tumor growth in vivo but regression occurs unimpaired and a relatively large turnover of DNA and proteins is still occurring in the growth arrested tumor. (b) RNA formed during this period falls within the standard classes known; however, the label incorporation into small RNA fractions (5 and 4S) is higher than the incorporation into larger RNA fractions. (c) Pulse-chase experiments suggest that initiation of regression speeds up the turnover rate of RNA. (3) We still do not have a clear idea of the role of RNA, if any, in the initiation of regression.

Significance to Cancer Research: (NCP Objective 6, Approach 6) Studies of events occurring during growth arrest and regression of mammary tumors in experimental models will contribute to the understanding of the human disease.

Proposed Course of Research: To continue to study the conditions of neoplastic tissue when regression starts, as an approach to understand the triggering mechanism of tumor destruction.

Keyword Descriptors: Mammary neoplasms, experimental; hormone-dependent tumor regression; dimethylbenz(α)anthracene-induced mammary tumor (rat); N-nitrosomethylurea-induced mammary tumor (rat); MTW9 mammary (rat); MtTW10 pituitary (rat); Actinomycin D; cycloheximide; DNA; RNA; animal models; pulse-labeling technique.

Honors and Awards: None

Publications:

1. Gullino, P.M., Pettigrew, H.M. and Grantham, F.H.: N-Nitrosomethylurea as mammary gland carcinogen in rats. J. Natl. Cancer Inst., 54: 512-516, 1975.

2. Butler, T.P. and Gullino, P.M.: Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. Cancer Res. 35: 512-516, 1975.

Project No. Z01 CBD 05211-04 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Initiation and Maturation Phenomena in the Replication of DNA

Previous Serial No.: NC-5211

Principal Investigator: William R. Kidwell

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2 1/4
Professional: 1
Other: 1 1/4

Project Description:

Objectives: Progression of the cell through the cell cycle is characterized by a series of events which occur in an ordered succession. Understanding how this ordered sequence is maintained is the objective of this research project. One specific focus is on the synthesis of poly(ADP-Ribose), a novel polymer occurring in eukaryotic nuclei. Accumulation of the polymer in cells appears to be maximal near the time of termination of DNA synthesis; that is, at the S/G_2 transition point. The mechanism by which polymer accumulation occurs and the role of the polymer in cell cycle progression is to be considered.

Methods Employed: HeLa cells were maintained in shake culture or in monolayers. Cell synchrony was achieved by blocking thymidylic acid synthesis de novo utilizing hydroxyurea. Poly(ADP-Ribose) polymerase activity in isolated nuclei was measured by the incorporation of ^{3}H-NAD into Poly(ADP-Ribose) which is insoluble in cold TCA. Quantitation of poly(ADP-Ribose) was performed by (1) conversion of the semi-purified material to PRAMP, a compound uniquely derived from the polymer followed by subsequent chromatographic identification or (2) direct quantitation utilizing a specific antibody to authentic poly(ADP-Ribose) using the FARR assay technique.

Major Findings:

Synthesis of poly(ADP-Ribose) in intact cells: Utilizing two independent assay methods, the synthesis of polymer has been shown to occur primarily near the S/G_2 transition point with a smaller amount synthesized in the last 1/3 of the S-phase. Agents which arrest cell cycle progression in early G_2 (adriamycin and macromomycin) also produce an accumulation of cells with elevated poly(ADP-Ribose) polymerase activity. The arrest of cell growth by neocarzinostatin and bichloroethylnitrosourea (which act late in G_2) is not associated with elevated polymerase activity. Thus the increase in polymer synthesis seen in vivo is shown to be due to a temporal increase in poly-(ADP-Ribose) polymerase near the S/G_2 transition point.

Poly(ADP-Ribose) Synthesis and Cell Division: Poly(ADP-Ribose) synthesis in normal HeLa cells is inhibited in vivo and in vitro by nicotinamide and by 5-methylnicotinamide. Both agents also block cell division. 5-methylnicotinamide has been utilized to select for variant HeLa cells whose growth is resistant to inhibition by this compound. Analysis of poly(ADP-Ribose) activity in nuclei from the normal and variant cells indicates that the enzyme from normal HeLa cells is more sensitive to inhibition by 5-methylnicotinamide than is the enzyme from the resistant cells. Thus there is apparently a causal relationship between the inhibition of poly(ADP-Ribose) synthesis and the inhibition of cell division. Unlike nicotinamide, 5-methylnicotinamide does not arrest normal HeLa cell growth during the DNA replicative phase. Rather, the analog causes these cells to accumulate somewhere between the end of the S-phase and the beginning of mitosis. Resistant cells continue to divide in the presence of 5-methylnicotinamide. This compound may be a specific inhibitor of poly(ADP-Ribose) polymerase since it has no effect on cellular NAD or ATP levels, both of which are altered by nicotinamide. These results are consistent with the hypothesis that poly(ADP-Ribose) synthesis is a prerequisite for cell progression to the mitotic events which follow G_2.

Significance for Cancer Research: (NCP Objective 6, Approach 3). Understanding the mechanisms whereby cell progression through the cell cycle is controlled may suggest ways in which cancer cells can be reversed to a non-transformed state, or killed. Poly(ADP-Ribose) synthesis may be a good model system for studying the transition from one growth phase to another. Synthesis of the polymer occurs predominantly at the S/G_2 transition and blockage of synthesis at this time correlates with the cells' failure to progress to mitosis.

Proposed Course: There are indications that the pattern of changes of poly-(ADPR) polymerase in continuously dividing cells differs from that seen in cells progressing from a growing to a resting state or in cells progressing from a growing to a regressing state. Is this merely a reflection of changes in the structure of the chromatin to which the polymerase is bound or is there a causal relationship? We will explore this possibility by simultaneously quantitating changes in the amount of polymer and polymerase activity during these transition times. As a model for growing to resting state transition we will utilize cultured lymphocytes stimulated by mitogens. A growing to regressing state test system will be that of hormonally responsive mammary cells or tumors in which regression is induced by withdrawal of hormone support. Continued effort will be made toward purifying and characterizing the enzymes involved in synthesizing and degrading poly(ADP-Ribose).

Keyword Descriptives: Poly(ADP-Ribose); cell division; G_2 blocking agents; transition point

Honors and Awards:

1. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. I. U.S. Patent Serial No. 3821087, June 28, 1974.

2. Knazek, R.A., Gullino, P.M., Dedrick, R.L, and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. II. U.S. Patent Application No. 441445, February 11, 1974.

3. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. Belgium Patent No. 799657, 1974.

4. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. E. Germany Patent No. 103925, 1974.

Publications:

1. Kidwell, W.R. and Burdette, K.E.: Poly(ADP-Ribose) synthesis and cell division. Biochem. Biophys. Res. Comm. 61: 766, 1974.

2. Kidwell, W.R.: Synthesis of poly(ADP-Ribose) during the G_2 stage of the cell cycle. J. Biochem. (Japan) (In press)

Project No. Z01 CBD 05216-04 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Cyclic Nucleotides on *In Vivo* Tumor Growth Inhibition.

Previous Serial No.: NCI 5216

Principal Investigator: Yoon Sang Cho-Chung

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2 1/2
Professional: 1
Other: 1 1/2

Project Description:

Objectives: Previous studies demonstrated that exogenous supply of cyclic nucleotides can inhibit *in vivo* growth of several but not all types of rat tumors. It was also found that the growth inhibition was not correlated with the elevation of intracellular cAMP level. Present studies were undertaken to investigate whether cAMP binding to receptor protein can be correlated with DBcAMP-induced tumor growth inhibition *in vivo.*

Methods:

1) Tumors:
Two cell populations isolated from Walker 256 carcinoma (hormone-independent mammary tumor) by selective transplantation; one regresses (DBcAMP-responsive) and the other continues to grow (DBcAMP-unresponsive) under DBcAMP treatment, were used.

2) Dibutyryl cAMP(DBcAMP) injections:
Injections of DBcAMP (10 mg/0.2 ml/NaCl/day/rat s.c.) were initiated when tumor size was about 3 g. Tumor growth was expressed as percent change in volume calculated from the formula, $4/3\ \pi r^3$, where r is 1/2 the average of the longest and shortest diameters measured daily.

3) cAMP assay: cAMP levels in tumors were measured by Gilman's competitive protein binding assay.

4) cAMP-binding assay:
   a) standard assay: cAMP binding activity of tumor cytosol (105,000 x g, 1 hr) was measured by the competition assay of Gilman at pH 4.5.
   b) total binding assay: the competition assay of Gilman was performed at 23° for 4 hr (^{3}H cAMP binding to proteins includes not only free sites but also endogenously bound sites by the exchange).

5) Adenylate cyclase assay: Activity of tumor homogenate was tested by the method of Krishra et al ± NaF.

6) cAMP-phosphodiesterase assay: Used the method of Thompson and Appleman at low and high-km substrate concentrations.

Major Findings:

1) cAMP-binding activity of DBcAMP-responsive and -unresponsive tumors after DBcAMP treatment *in vivo*:

   When responsive tumor is stimulated with DBcAMP injection of host, there is a rise in the amount of receptor proteins endogenously saturated by cold cAMP as indicated by decrease in their ability to bind exogenously added [^{3}H] cAMP at standard assay condition (see method). Pretreatment of tumor bearing animal with cycloheximide abolished this depression but actinomycin D did not show any influence. On the contrary, the binding activity of unresponsive tumor cytosol remained low without change despite elevation of cAMP level. Thus impaired saturation of cAMP binding protein *in vivo* was found in unresponsive tumor.

2). Specific cAMP-binding activity of tumor cytosol *in vitro*: Specific cAMP-binding (at standard condition) by tumor cytosol at various concentration of [^{3}H] cAMP indicated that there is considerably less specific cAMP-binding by unresponsive tumor cytosol. Scatchard analysis showed 10 fold higher equilibrium dissociation constant (Kd) for unresponsive tumor cytosol. Thus quantitatively and qualitatively deficient cAMP-binding activity was found in unresponsive tumor.

3) Effect of prostaglandin E_1 (PGE_1) on the cAMP system of DBcAMP-responsive and -unresponsive tumor slices *in vitro*: i) significant induction of basal and NaF-stimulated adenylate cyclase and cAMP-phosphodiesterase (low Km) was found only in responsive tumor slices incubated at 30°, for 10 min with PGE_1. ii) significant elevation (20 fold over basal) of cAMP level in responsive tumor slices incubated at 30°, for 30 min with PGE_1 indicated the accumulation of cAMP mainly due to adenylate cyclase induction, while only small elevation of cAMP level found in unresponsive tumor slices after the

incubation was due to the inhibition of phosphodiesterase by theophylline added in the incubation medium. iii) cAMP-binding activity: PGE_1 significantly increased maximum total binding activity (see the method) of responsive tumor slices but did not influence binding activity of unresponsive tumor. Furthermore the binding assay curve indicated in vitro instability of cAMP-binding by unresponsive tumor cytosol. Thus impaired cAMP metabolism of DBcAMP-unresponsive tumor in vitro reflects its behavior in vivo.

Significance to Cancer Research: (NCP Objective 6 Approach 3) These studies contribute to the understanding of the mechanism of cyclic nucleotide-induced tumor regression. The coordinated activation of cAMP system elicited by PGE_1 in DBcAMP-responsive tumor suggests that there must be a key regulator which turns on and off cAMP system. The finding of impaired cAMP-binding activity in correlation with DBcAMP-unresponsiveness of experimental tumor can be utilized for the identification of DBcAMP-responsiveness in clinical applications.

Proposed Course of Research: To extend the investigations on the mechanism of cAMP action on tumor growth inhibition, the following proposal is made:

1) Characterization of purified cAMP-binding protein in correlation with DBcAMP-responsiveness.

2) Assess whether instability of cAMP-binding activity, in vitro can be utilized as a marker of DBcAMP-unresponsiveness.

3) Investigate whether cAMP-binding protein has any relation with regulatory subunit of adenylate cyclase (purification of adenylate cyclase is necessary for the ultimate results).

4) Investigate NaF and hormonal stimulation of adenylate cyclase in DBcAMP-responsive and -unresponsive tumors.

5) Investigate DBcAMP-responsiveness in relation to cGMP system.

6) Investigate cAMP and cGMP system in hormone-dependent mammary tumor regression, induced by hormonal deprivation.

Keyword Descriptors: Mechanisms of cyclic nucleotide action; tumor growth inhibition in vivo.

Honors and Awards: None

Publications:

1. Cho-Chung, Y.S.: In vivo inhibition of tumor growth by cyclic adenosine 3',5'-monophosphate derivatives. Cancer Res. 34: 3492-3496, 1974.
2. Cho-Chung, Y.S. and Berghoffer, B.: The role of cyclic AMP in neoplastic cell growth and regression. II. Growth arrest and glucose-6-phosphate dehydrogenase isozyme shift by DBcAMP. Biochem.Biophy. Res.Comm. 60:528-534, 1974.

Project No. Z01 CBD 05219-04 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In Vitro Simulation of Hormone-dependent Mammary Tumor Regression

Previous Serial No.: Same

Principal Investigator: R. A. Knazek

Other Investigator: P. M. Gullino

Cooperating Units: None

Man Years:

Total: 1 1/2 + 1 Stride
Professional: 1/2
Other: 1 + 1 Stride

Project Description:

Objectives: To reproduce and study in vitro the hormone-dependent regression process of mammary tumors observed in vivo.

Methods Employed: The MTW9 rat mammary carcinoma will grow in vivo only when a prolactin/growth hormone-producing tumor is concomitantly present in the host animal. Removal of the pituitary tumor causes rapid regression of the mammary carcinoma, providing an excellent model for hormone-dependent human mammary carcinoma.

Medium conditioned by pituitary tumor cells and supplemental hormones is used to support the growth of MTW9 mammary carcinoma in vitro in both monolayer and artificial capillary cultures. The latter permits mammary carcinoma to be studied under specific in vitro conditions that very closely simulate the in vivo state without the uncontrollable in vivo variables.

An assay for prolactin receptors on the membrane of the MTW9 cell is being developed using modifications of the Shiu-Friesen technique.

Major Findings: Rat pituitary tumor cells form solid tumors within the artificial capillary culture units and produce in excess of 100 μg prolactin per day. Various types of capillaries and combinations thereof have been used to grow rat pituitary tumors. These studies indicate that both oxygen and macromolecular transport are limiting factors in cell growth and hormone secretion. For this reason, nutrient capillaries permeable to substances of up to 100,000 MW are mixed with a fixed ratio silicone polycarbonate oxygenating capillaries within a polycarbonate shell to form the standard capillary culture unit.

Initial observations indicate that cells obtained from an MTW9 tumor can be maintained within an artificial capillary network perfused with medium that has been conditioned by a pituitary tumor growing within another capillary unit upstream of the target mammary culture.

Medium conditioned by pituitary tumor cells causes increased thymidine uptake by MTW9 mammary carcinoma during the first week in culture.

Significance for Cancer Research: (NCP Objective #4, Approach #1). The environment which artificial capillaries provide is more physiologic than that of any other type of cell culture. This allows isolated cells to form solid tumor masses *in vitro* and to exhibit behavior that more closely resembles that of the *in vivo* state. Thus, solid mammary tumors may be formed *in vitro* and the hormone-dependent regression process can be studied without uncontrollable *in vivo* factors.

Knowledge of the effect of various hormones on prolactin receptors and their subsequent role in the growth and regression processes may reveal the mechanisms involved and perhaps provide information that can be used to induce clinical improvement of patients with breast cancer.

Proposed Course of Research: The effect of prolactin, growth hormone, insulin, and steroids upon the prolactin receptor and the subsequent behavior of the MTW9 tumor will be studied in both the *in vivo* and *in vitro* situations.

Keyword Descriptors: Breast cancer, *in vitro*, regression, hormone, prolactin, receptor, MTW9, pituitary tumor, capillaries, cell culture

Honors and Awards:

1. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes, I. U.S. Patent 3821087, 1974.

2. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes, II. U.S. Patent Application 441445, 1974.

3. Knazek, R. A., Guest Lecturer, Department of Medicine, Massachusetts General Hospital, Boston, Mass., January 1975.

4. Knazek, R.A., Visiting Faculty Member at the W. Alton Jones Cell Science Center, "Perfusion Culture Systems," Lake Placid, N.Y., April 8-10, 1975.

5. Knazek, R.A., Guest Lecturer, College of Dentistry, University of Toronto, Toronto, Ontario, June 1975.

6. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. Belgium Patent No. 799657, 1974.

7. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. E. Germany Patent No. 103925, 1974.

Publications:

1. Knazek, R.A.: Solid tissue masses formed *in vitro* from cells cultured on artificial capillaries, Fed. Proc. 33: 1973, 1974.

Project No. Z01 CBD 08205-04 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Application of the Capillary Culture Technique to (a) Virus Production (b) Bone Marrow Culture (c) Urokinase Synthesis and (d) Polypeptide Hormone Production

Previous Serial No.: Same

Principal Investigators:
a) R. A. Knazek, W. R. Kidwell, P. Qasba, and P. M. Gullino
b) R. A. Knazek and M. J. Cline
c) R. A. Knazek and J. J. Pisano
d) R. A. Knazek and J. S. Skyler

Other Investigators: None

Cooperating Units:
b) Hematology Division, Department of Medicine, UCLA
c and d) Experimental Therapeutics Branch, NHLI

Man Years:

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives:

(a) Development of a method that provides continuous and/or improved production of virus particles in vitro.

(b) Growth and study of normal bone marrow in vitro.

(c) Development of a method to provide continuous production of large quantities of human urokinase.

(d) Development of a method that provides both large quantities of pituitary hormones and a means for studying their synthesis and secretion.

Methods Employed: (a) Established or primary mammalian cell suspensions are injected into the extracapillary space of an artificial capillary culture unit. When the capillaries are perfused with tissue culture medium these cells grow to multilayer depths at which time they are infected with a small inoculum of virus. The viral progeny are removed from the extracapillary space periodically during the subsequent weeks of culture. (b) Aspirates of normal bone marrow are injected into the extracapillary space of an artificial capillary culture unit. The capillaries are then perfused with oxygenated tissue culture medium which provides a continuous source of nutrients and enables cell product removal by diffusion without disturbing the pericellular environment of the extracapillary space. Stimulatory macromolecules of high molecular weight are inoculated with the cell suspension but do not diffuse through the capillary walls. Thus, the cells are provided with a high concentration of poetic factor and a large nutrient/product reservoir. (c) Urokinase is currently used clinically in the treatment of thrombotic events. This enzyme is obtained commercially from human fetal kidney cells (HFK) maintained in monolayer forms requiring rather cumbersome and expensive techniques. This project consisted of culturing HFK cells within the artificial capillary culture units to collect urokinase as it is secreted into the perfusion medium. (d) Human pituitary tumors are inoculated into the capillary culture units and maintained by continual recirculation and frequent replacement of perfusion medium; subsequently, the hormone is extracted from the perfusate for chemical or biologic studies.

Major Findings: (a) Initial studies indicated that Vero cells grown within the capillary culture unit and then infected with SV-40 would produce small amounts of additional virus. However, in subsequent experiments infection of capillary units, heavily overgrown with cells did not produce any detectable new virus. (b) Granulocytes, monocytes, erythrocytes, and megakaryocytes can all be maintained in artificial capillary culture units for two weeks during which they divide and grow. The percentage of viable cells at this time was approximately twice that obtained using standard marrow culture methods. Although granulocytic and erythrocytic cell lines responded to colony stimulating factor and erythropoetin, megakaryocytes did not respond to a thrombopoetic concentrate. Cells removed from the extracapillary space of the culture unit exhibit progressive loss in their ability to form colonies with agar culture: This implies loss, with time, of the pluripotentiality of the stem cells initially present in the inoculum. (c) None. (d) A human pituitary tumor maintained within a capillary culture unit secreted nearly 3 mg prolactin during its first 4 months of culture and continued to secrete significant amounts 3 months later. This culture technique now permits physiologic kinetic studies of prolactin release and synthesis that have not been possible with other methods: An 8 minute lag time is required before thyrotropin releasing hormone can induce the expected massive burst of stored prolactin released into the medium followed by a less massive secretion rate that remains above base levels for two hours.

Significance for Cancer Research: (NCP Objective #3, Approach #3).

(a) A technique that provides a convenient and efficient method for producing large quantities of virus would be of value for both viral studies and vaccine production.

(b) All mature blood cell components may now be maintained in vitro. Study of their metabolism and growth characteristics will provide further insight into the behavorial controls of the hematopoietic system, both in normal and neoplastic states. The possibility also exists for a patient's bone marrow to be aspirated and maintained or grown in vitro to serve as a tissue replacement source after chemotherapy has depressed its marrow. Neoplastic marrow may also be maintained in nearly physiologic condition to study its response to different chemotherapeutic agents and programs.

(c) Development of a technique that permits diploid cells to remain differentiated both morphologically and functionally would be a valuable laboratory tool.

(d) Human pituitaries cultured within artificial capillary units may provide significant amounts of prolactin or other hormones for studies of hormone-dependent breast cancer. The construction of the culture device permits study of rapid responses to both stimuli and inhibitors of cell function and may provide further insight into the mechanism of hormone synthesis and secretion.

Proposed Course of Research:

(a) Non-lytic viral systems will be studied to determine if the improved or continuous viral yields are feasible using this technique.

(b) Capillary bundles of various types and conformations will continue to be studied to provide optimum conditions for hematopoesis.

(c) Studies on urokinase have been terminated because of difficulty in growing large quantities of diploid cells and performing urokinase assays.

(d) Stimulation and inhibition of prolactin and growth hormone will be studied using human pituitary tumors cultured within arterial capillary units.

Keyword Descriptors: Capillary culture; cell culture; virus; bone marrow; growth hormone; prolactin.

Honors and Awards: None

Publications:

1. Knazek, R.A. and Skyler, J.S.: Production of human prolactin by pituitary adenemata grown on artificial capillaries. (Abstract), Endocrine Society, 1975.

2. Skyler, J.S., Knazek, R.A., Rogol, A.D., and Lovenbert, W.: Characterization of growth hormone and prolactin produced by human tumor in culture. (Abstract), ASCI, 1975.

3. Knazek, R.A. and Skyler, J.S.: Production of growth hormone and prolactin in tissue culture. (Abstract) International Symposium on Growth Hormone and Related Peptides, Milan, Italy, 1975.

Project No. Z01 CBD 08206-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Cyclic AMP-dependent Protein Kinase in Tumor Growth Inhibition *In Vivo*

Previous Serial No.: None

Principal Investigator: Pon-Nyong Yi

Other Investigator: Yoon Sang Cho-Chung

Cooperating Unit: None

Man Years:

Total: 1 1/4
Professional: 1 1/4
Other: 0

Project Description:

Objectives: The participation of cAMP-dependent protein kinases in the biological actions of cAMP is well established in a variety of tissues and species. Present studies were undertaken to investigate the role of protein kinase in dibutyryl cAMP-induced tumor growth inhibition, *in vivo*.

Methods Employed:

1) Tumors: Two cell populations isolated from Walker 256 carcinoma (hormone-independent mammary tumor) by selective transplantation, one regresses (DBcAMP-responsive) and the other continues to grow (DBcAMP-unresponsive) under DBcAMP treatment, were used.

2) Protein kinase assay: The enzyme activity was determined by measurement of the incorporation of ^{32}P from α-labeled ATP into histone. Incubations were at 30° for 5 min with or without 1 μM cAMP and the reaction terminated by 10% TCA. The protein precipitate collected on glass fiber filters (millipore) was washed with 10% TCA and counted.

3) DEAE-sephadex A-50 and sephadex G-200 column chromatography were used to separate inactive (CR) and active (C) protein kinases.

Major Findings:

1) cAMP-activation of protein kinase in DBcAMP-responsive and -unresponsive tumors: The histone phosphorylation with tumor cytosol was activated by cAMP. The cAMP activation of protein kinase of DBcAMP-responsive tumor was 60% while that of -unresponsive tumor was 100%. The lower cAMP-activation of the kinase in responsive tumor reflected in its higher cAMP-independent kinase activity.

2) Kinetic properties of protein kinase: The same pH optimum of 7.5 and the Km of $1.5x10^{-5}M$ for ATP was found with the kinase of both DBcAMP-responsive and -unresponsive tumors. This indicates that the catalytic subunits of protein kinase in responsive and unresponsive tumors are probably similar if not identical.

3) Separation of inactive (CR) protein kinase from active (C) protein kinase: Chromatography of tumor cytosol on either DEAE-sephadex or sephadex G-200 columns resulted in the separation of inactive (CR) protein kinase from active (C) protein kinase and showed cAMP-binding macromolecules migrated with the inactive (CR) protein kinase.

4) Effect of Prostaglandin E_1 (PGE_1) on protein kinase of DBcAMP-responsive and -unresponsive tumors: Incubation of responsive tumor slices with PGE_1, at 30°, for 10 min in vitro, resulted in a significant increase of both cAMP-dependent and independent protein kinase activity while the same incubation of unresponsive tumor slices with PGE_1 showed only small increase of cAMP-independent activity without influencing the cAMP-dependent activity. Thus, PGE_1 increased % cAMP stimulation of protein kinase in responsive tumor slices while decreasing the % stimulation in unresponsive tumor slices.

Significance to Cancer Research: (NCP Objective 6, Approach 3). These studies contribute to understanding the relationship between dibutyryl-cAMP induced in vivo tumor growth inhibition and cAMP-dependent protein kinase. The significant increase of cAMP-dependent protein kinase (CR) activity only in DBcAMP-responsive tumor slices by PGE_1 indicated a correlation between DBcAMP-responsiveness and regulatory subunit of protein kinase (cAMP-binding protein) was shown to be similar.

Proposed Course of Research: To study the role of protein kinase on the inhibition of tumor growth in vivo, the following proposal is made:

1) Separation and purification of inactive (CR) and active (C) protein kinase.

2) Separation of inactive (CR) protein kinase into its subunits, namely catalytic (C) and regulatory (R) components and reconstitution of CR by interchanging the subunits of responsive and unresponsive tumor protein kinases.

3) Determine whether cellular protein phosphorylation (especially, histone or microsomal protein) is linked with cAMP action on tumor regression.

Keyword Descriptors: Protein kinase; cAMP-dependent protein kinase; Walker 256 rat mammary carcinomas, responsive and unresponsive to DBcAMP; DBcAMP treatment of Walker 256 rat mammary carcinoma.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 08207-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation of Tumor Temperature.

Previous Serial No.1: None

Principal Investigator: Pon-Nyong Yi

Other Investigators: Pietro M. Gullino

Cooperating Units: None

Man Years:

Total: 1/2

Professional: 1/2

Other: 0

Project Description:

Objectives:

1) To compare the temperature of the solid tumor in normal and anesthetized rats.

2) To measure oxygen and glucose consumption and lactate production when the temperature of the tumor is either increased or decreased.

3) To assess the degree of metabolic impairment produced by changes of temperature in the tumor *in vivo*.

Methods Employed: In the same rat two YSI thermisters were placed subcutaneously in the left and right lumbar region, respectively. Few fragments of W256 or MTW9 mammary carcinomas were placed aroung the thermisters of the right lumbar region. After a few days a solid tumor incorporated the thermister and the temperature of the tumor could be compared with that of the same region in the same rat. Oxygen, glucose, and lactate were measured by standard techniques.

Major Findings: Project No. Z01 CBD 08207-01 LPP

1) In the normal animal kept in a restraining cage at ambient temperature of 25 C, the subcutaneous temperature reads 36 C and tumor temperature 37 C. This difference remains constant over 24 hrs without apparent diurnal rhythm.

2) In the anesthetized animal tumor temperature becomes lower than the subcutaneous temperature; the level of the depression depends on the anesthetic.

3) When the anesthetized animal is warmed to 42 C, tumor and subcutaneous temperatures become equal within 10 minutes and close to 42 C. From this level, as the animal is cooled, tumor temperature decreases more rapidly than subcutaneous temperature and also reaches lower levels before climbing to the standard difference as indicated in 2).

4) Lower temperatures upon cooling were also observed in tumors of anesthetized animals.

5) The influence of changing temperature on tumor metabolic rates is under study and results are not yet available.

Significance to Cancer Research: (NCP Objective 6, Approach 7). Hyperthemia has been used to destroy solid tumors. The physiopathological bases of this destruction are poorly known and this project will improve them.

Proposed Course of Research: To complete the experiments needed to answer the questions reported under objectives.

Keyword Descriptors: Mammary tumors, experimental; temperature; metabolism; biophysical techniques.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 08208-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of Nuclear Proteins in the Regression of Hormone-dependent Mammary Tumors MTW9.

Previous Serial No.: None

Principal Investigator: Maurice Rouleau

Other Investigator: Pietro M. Gullino

Cooperating Units: None

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: To assess whether nuclear proteins of the MTW9 mammary tumor, in particular the acidic ones, show changes in quality and quantity due to hormone deprivation of the host.

Methods Employed: Double-isotope labeling of tumor proteins in vivo. ^{14}C-protein hydrolyzate is injected intraperitoneally in the growing tumor bearing animals. A ^{3}H-hydrolyzate is injected intraperitoneally to the regressing tumor bearing animals. After 1 hour, the tumors are removed and pooled, the isolated nuclei are extracted and the nuclear proteins analyzed by polyacrylamide gel electrophoresis. Any change in the $^{14}C/^{3}H$ ratio for a particular protein will indicate some modifications following hormonal withdrawal.

Major Findings: One hour after castration, the ratio $^{14}C/^{3}H$ remains the same for all the nuclear proteins indicating no difference between the growing and the regressing mammary tumors. This is probably due to the presence of hormones in the circulating blood after such a short period of time after castration. Analysis of tumors removed at later times is underway.

Significance to Cancer Research: (NCP Objective 6, Approach 3) These studies will contribute to the understanding of the biochemical mechanisms underlying the phenomenon of tumor regression.

Such an understanding of the conditions which induce regression of experimental mammary tumors will help to elucidate the mechanism of growth arrest or regression in human mammary tumors, and improve possibilities for rational therapy.

Proposed Course of Research: To extend these studies at different times after castration, i.e. when the level of circulating hormones in the castrated animal is nearly zero.

Keyword Descriptors: Nuclear proteins; MTW9 mammary tumor; hormonal deprivation.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 08209-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Synthesis and Processing of Heterogeneous Nuclear RNA in Non-dividing Human Lymphocytes and Modifications Related to Stimulation of Cell Growth.

Previous Serial No.: None

Principal Investigator: Herbert L. Cooper

Other Investigator: Shelby L. Berger

Cooperating Units: None

Person Years:

Total: 3/4
Professional: 1/2
Others: 1/4

Project Description:

Objectives: To characterize the synthesis and processing of heterogeneous nuclear RNA, thought to be the precursor to messenger RNA, in physiologically non-dividing human cells. Points in the metabolic sequence which may be involved in control of normal processing will be sought. The relation between such biochemical controls and modifications in the use of messenger RNA related to growth stimulation will be sought. Comparisons will be made between the functioning of these regulatory steps in normal and neoplastic lymphoid tissues.

Methods: Routine procedures used throughout.

Major Findings: In this phase of the project, the synthesis, molecular size distribution and turnover characteristics of hnRNA were examined in non-dividing cells. It is widely believed that large hnRNA molecules are synthesized in the nucleus, following which a sequence of up to 200 adenylates is added to the 3'-terminus. A shortening process follows, during which most of the 5'-end of the molecule is degraded. The remaining product is transported to the cytoplasm as mRNA.

We observed that the largest hnRNA molecules were poorly represented in the pool of poly(A) bearing hnRNAs. Previous work showed that these large molecules were very rapidly turned over. In the present study, we found that poly(A)-bearing hnRNA turns over much more slowly, with a half-life of 7 hours. Furthermore, during this processing period, the average size of hnRNA(+)poly(A) molecules remained unchanged. This indicated that the processing of larger hnRNA(+)poly(A) molecules to smaller mRNAs takes place by a rapid conversion, probably a single endonucleolytic cleavage, without formation of intermediate-sized forms. During the decay of hnRNA(+)poly(A) it was estimated that more than 60% of the hnRNA molecules left no detectable mRNA product. From this we conclude that a large proportion of potential mRNA in resting lymphocytes is either never successfully converted to mRNA, or the mRNA product is highly labile. The latter possibility is supported by the results of another project in progress in this laboratory (#08212). However the former possibility is not eliminated.

These results suggest at least two sites of regulation in the processing of hnRNA which may permit selection of mRNAs for translation in terms of the physiological requirements of the cell:

1). The decision to polyadenylate new hnRNAs may select between pre-mRNAs which will be rapidly degraded and those which will enter a pool of longer-lived hnRNA(+)poly(A) from which particular mRNAs may be withdrawn as required.

2). The slow decay of hnRNA(+)poly(A) offers the possibility of selection of particular messages for further processing according to momentary needs of the cell. Messages not needed are eventually degraded without processing.

Significance for Cancer Research: This project conforms to Objective #3, Approach #1 of the National Cancer Plan. It aims at an understanding of the biochemical control mechanisms by which normal cell growth and function are maintained. The causes of disordered cell growth and activity in neoplasia may then be better understood and rational attempts made to prevent or modify them.

Proposed Course of Research: Examination of changes in hnRNA processing related to cell growth stimulation will next be studied. Following this, a comparison will be made between growing lymphocytes from normal human peripheral blood and from neoplastic lymphoid cells. The latter will include long term lymphoid lines (Burkitt lymphoma, e.g.) of both B- and T-cell types; patient material from chronic lymphocytic leukemia, lymphomatous lymph node cells, and, if possible, acute lymphatic leukemia.

Keyword Descriptors: Lymphocytes; resting cells; heterogeneous nuclear RNA; poly(A)-bearing RNA.

Honors and Awards: None

Publications: The results of this study have been submitted for publication.

Project No. Z01 CBD 08210-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Behavior of Ribosomes, Polysomes and Ribosomal Subunits in Non-dividing Human Cells and Changes Related to Onset of Normal Cell Growth.

Previous Serial No.: None

Principal Investigator: Herbert L. Cooper

Other Investigator: Shelby L. Berger

Cooperating Units: None

Person Years:

Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives: To characterize the behavior of ribosomal particles in a non-growing cell population in which the rate of protein synthesis is maintained at low levels; to correlate such behavior with the limitation in protein synthesis so as to understand the mode of translational control employed by the cell in adjusting protein synthesis to growth and metabolic activities; to determine the regulatory steps which are operative in the enhancement of protein synthesis associated with the onset of cell growth.

Methods: In addition to routine methods, techniques were developed to permit examination of ultraviolet absorbency profiles of ribosomes and polysomes from resting and growing human lymphocytes analyzed by ultracentrifugal sedimentation in sucrose gradients. Because of limited amounts of material, and the small size of lymphocytes, this methodology has previously never been successfully applied to human peripheral blood lymphocytes. We overcame these problems by a combination of optimizing lymphocyte yields from human peripheral blood, and a detergent cell lysis method which gave maximum yields of cytoplasm while minimizing degradation of ribosomes.

Major Findings: Unlike growing cell systems studied by most investigators, physiologically resting lymphocytes were found to maintain the great majority (about 70%) of their cytoplasmic ribosomes as single 80s ribosomes, monomers. In addition, significant numbers of unassociated (native) 60s and 40s, subunits were found.The monomers were found to consist of a major fraction (80%) of inactive particles (easily dissociable in high salt, "free ribosomes") and a minor fraction of active particles (monosomes). A major portion of the protein synthesis of the resting lymphocyte was found to occur on such monosomes (i.e. - a single ribosome traversing a mRNS), while free ribosomes did not engage in protein synthesis. Progressively decreasing numbers of larger polysomes were found. The number of native subunits was large relative to the numbers of polysomes. The large accumulation of free ribosomes (i.e. - associated subunits inactive in protein synthesis) was shown to be a real feature of the intact lymphocyte and not an artifact due to association of large pools of free subunits during technical procedures. Thus, the low rate of protein synthesis in resting lymphocytes is correlated with:

1). Maintenance of most of the cell's available ribosomes in an inactive state (free ribosomes).

2). Performance of protein synthesis largely on monosomes and smaller polysomes, with inefficient use (i.e. - unsaturation) of available mRNA.

Labeling studies with [^{3}H] uridine revealed that new rRNA first entered native 40s and 60s subunits and polysomes (including monosomes). As particles aged, they accumulated in the pool of inactive free ribosomes. There was no rapid interchange between the older subunits in free ribosomes and the newer native subunits. We interpret these findings as follows:

New ribosomal subunits spend a period during which they cycle between the pools of dissociated native subunits and the polysomes and monosomes, participating in protein synthesis. Since the first events in initiation of protein synthesis are currently believed to occur on dissociated ribosomal subunits, this cycling of active ribosomes requires 80s ribosomes to dissociate into subunits before re-initiation can occur. Our evidence suggests that, in resting lymphocytes, after a period of activity, ribosomes fail to dissociate, and therefore become inactive. Failure to dissociate may result from absence of a "dissociation factor" which has been postulated to exist in other systems. Thus, physiological limitation of protein synthesis in resting lymphocytes may be controlled, in part, through restriction of the availability of a ribosome dissociation factor, thereby limiting the number of ribosomes which can be active at any moment.

Following growth stimulation with phytohemagglutinin (PHA), inactive free ribosomes were activated to engage in protein synthesis. This was detected easily at 2 hrs and reached a peak at 14-16 hrs, when virtually no inactive free ribosomes remained. However, the great majority of the now active ribosomes remained as monosomes at this time, indicating persistence of the condition of unsaturation of mRNA.

As growth stimulation progressed, the average size of polysomes increased and the proportion of monosomes diminished. However, the numbers of inactive free ribosomes also began to increase again after 20 hrs, until they became a major feature once again.

We interpret this sequence as follows:

1). In the resting state, protein synthesis is limited by the sequestration of ribosomes due to restriction in a dissociation factor.

2). During the onset of growth, inactive ribosomes are recruited by a rapid increase in dissociation factor. For a number of hours, these ribosomes do not saturate the available mRNA, probably because of an excess of mRNA relative to ribosomes.

3). As the most active rate of cell growth is reached, dissociation factor once more becomes limiting, and inactive free ribosomes reaccumulate. At this time, mRNAs are more effectively saturated, with larger polysomes formed. Therefore, the numbers of active ribosomes are no longer limiting. Instead at this late stage, the availability of mRNA appears to be the limiting factor in regulating protein synthesis.

Significance to Cancer Research: By the elucidation of the mechanisms which regulate protein synthesis in resting cells and during a physiological growth sequence, we hope to provide the basis for recognition and understanding of derangements in these controls which may characterize malignant transformation. This objective supports Objective 3-1 of the NCP.

Proposed Course of Research:

1). Further characterizaiton of the basis for inactivation and re-activation of ribosomes in resting lymphocytes.

2). Examination of sequential events in this system during the return to resting state which occurs *in vitro* following stimulation by PHA.

3). Investigation of the possibilities for influencing control steps which have been elucidated by the use of drugs or other approaches. *In vitro* studies only at this stage.

4). Comparison of normal and neoplastic lymphoid cells with respect to these control mechanisms.

Keyword Descriptors: Lymphocytes; resting cells; ribosomes; polysomes; cell growth stimulation.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 08211-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Detection of Specific Messenger RNAs in Resting and Growing Human Lymphocytes.

Previous Serial No.: None

Principal Investigator: Herbert L. Cooper

Other Investigator: Shelby L. Berger

Cooperating Units: None

Person Years:

Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives: To determine whether mRNAs for specific products synthesized only by growing lymphocytes are present but untranslated in resting lymphocytes; to determine whether such sequences are present in the nucleus, cytoplasm, or both.

It is hoped, by this approach, to clarify the level of control of the source of mRNA for specific proteins, and the way in which growth stimulation affects these controls.

Methods: RNA will be extracted from resting and growing lymphocytes (whole cells, cytoplasm, and nuclei). Messenger RNA will be separated by affinity chromatography on oligo deoxythymidylate cellulose; mRNA will then be injected into amphibian oocytes. Proteins synthesized by injected and control oocytes will be examined for activity of specific proteins. Initially, assays will be performed for human interferon. This protein is produced only by growing lymphocytes, and can be assayed in minute amounts. Since it is species specific, no interference from amphibian proteins is anticipated.

Major Findings: This project has not yet begun, but will be initiated in the current fiscal year.

Significance to Cancer Research: Supports Objective 3-1, 3-2, and 3-3 of NCP.

Proposed Course of Research: As indicated above.

Keyword Descriptors: Lymphocytes; messenger RNA

Project No. Z01 CBD 08212-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control of Messenger RNA Metabolism in Human Lymphocytes

Previous Serial No.: None

Principal Investigator: Shelby L. Berger

Other Investigator: Herbert L. Cooper

Person Years:

Total: 1 1/2
Professional: 1/2
Others: 1

Project Description:

Objectives: To elucidate the steps involved in the synthesis and degradation of messenger RNA and its precursors; to determine the mechanisms employed by the cell in exercising control at key steps in mRNA metabolism and to devise methods of inhibiting or activating these processes in order to manipulate cell growth.

These objectives pertain to non-dividing and actively proliferating normal human lymphocytes and to neoplastic lymphoid tissues.

Methods: Routine procedures have been used throughout. The use of diethyl pyrocarbonate, a potent nuclease inhibitor, made possible reproducible isolation of lymphocyte poly(A)-bearing RNA on columns of oligo(dT) cellulose. This inhibitor has also allowed us to regenerate the same columns for use indefinitely without fear of ribonuclease contamination.

Major Findings: The lymphocyte is ideally suited for studies of the complex mechanisms by which resting cells enter a state of active proliferation or return from a growing state to a condition of restricted cellular activity. When isolated from human peripheral blood, the lymphocyte is a non-growing cell. However, it can be stimulated at will to enter a period of rapid growth by the addition to the medium of one of a number of mitogens.

Messenger RNA (poly(A)-bearing RNA) life times were studied by incubating cells for a short period of time with radioactive RNA precursors, washing them and resuspending the cells in unlabeled medium. After any desired interval, the amount of poly(A)-bearing RNA (mRNA) in the cytoplasm can be determined. When such experiments were performed with resting lymphocytes, two classes of cytoplasmic poly(A)-bearing RNA were identified. A labile class with a half-life of less than 17 minutes could be discerned between 2 and 3 hours of chase incubation. Thereafter a stable component of mRNA was detected which did not appear to be degraded throughout 24 hours of observation. The size distribution of both newly synthesized and stable mRNAs were determined by gel electrophoresis. Each class was heterogeneous with a peak distribution in the molecular weight range of 2.5 to 3.5 x 10^5. This size is characteristic of messenger RNA. However, the newly synthesized poly(A)-bearing RNA (which includes labile and stable messages) had a slightly broader size distribution and was on the average slightly larger than the stable variety.

The mechanism by which messages are sorted into stable and labile categories has not yet been determined. Since it is unlikely that shorter messages are per se more useful to the cell and therefore more worthy of being conserved than the longer, labile variety, our findings can be interpreted as evidence for a cytoplasmic step in the processing of all mRNAs. The apparent shortening of messages probably includes loss of nucleotides from the 5'-end as well as loss of adenylate residues from the poly(A) "tail." The functional significance of this observation is also unknown.

Nevertheless, the existence of two species of messenger RNA in the resting cell and of messages with different lifetimes in the growing cell provides a potential means for the posttranscriptional regulation of protein synthesis by controlling the supply of specific messages.

Significance for Cancer Research: Objective 2, #3; Objective 3, #1,3 and 4.

Proposed Course of Research: The basis for the classification of messenger RNAs into stable and labile categories shall be pursued in the near future. Stabilization of certain messages may be accomplished by several mechanisms among which are: (1) binding of the mRNA to a protective protein; (2) specific chemical modification of the RNA molecule itself; (3) combination with the protein synthesizing apparatus of the cell. Each of these possibilities shall be investigated. If indeed there are mRNA protecting proteins, we shall characterize those associated with both stable and labile messages. This project will require the development of adequate methods to isolate RNA-protein complexes from lymphocytes without massive degradation of RNA. The sequences in both classes will be compared by hybridization studies and the encoded protein products studies. (See Project #08211.)

The role of cytoplasmic processing of messenger RNAs will also be examined. We hope to elucidate the nature of the shortening reaction, and perhaps identify the enzyme(s) involved.

The study of labile and stable messenger RNAs in malignant lymphoid cells is anticipated.

Keyword Descriptors: Lymphocyte; mRNA half-life; poly(A)-bearing RNA

Honors and Awards: None

Publications: Berger, S.L.: Diethyl Pyrocarbonate: An examination of its properties in buffered solutions with a new assay technique. Anal. Biochem. 1975 (In press)

Project No. Z01 CBD 08213-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Glucose-6-Phosphate Dehydrogenase Isozymes in Growing and Regressing MTW9 Rat Mammary Carcinomas.

Previous Serial No.: None

Principal Investigator: Jeffrey S. Bodwin

Other Investigators: Yoon S. Cho-Chung

Cooperating Units: None

Man Years:

Total: 2

Professional: 2

Others: 0

Project Description:

Objectives: Previous work from this laboratory has shown that G6PD enzyme activity of rat mammary carcinomas exists in 4 isozymic forms located in mitochondrial, microsomal and cytosol cell fractions. Treatment of tumor-bearing rats with DBcAMP resulted in tumor growth arrest and disappearance of tumor microsomal G6PD activity. Further characterization of G6PD isozymes is pursued to elucidate their role as biological markers in the early event of tumor regression.

Methods Employed:

1) Primary 7,12-DMBA induced and MTW9 transplantable rat mammary tumors, both hormone-dependent, were used in studies.

2) G6PD activity of tumor extract was assayed directly with a spectrophometer method and also histochemically by staining acrylamide gels after electrophoresis.

3) Partial purification of enzymes was performed with preparative disc electrophoresis.

Major Findings:

1) MTW9 carcinomas regressing after hormonal deprivation of the host show a decrease in G6PD activity due to reduction in microsomal and soluble enzymes.

2) Treatment of MTW9 tumor-bearing animals for 3 days with DBcAMP resulted in tumor growth arrest and microsomal G6PD activity disappearance. Cessation of treatment restores tumor growth and microsomal activity.

3) Castration of 7,12-DMBA tumor-bearing rats results in marked decrease in soluble G6PD activity.

4) Glucose-6-phosphate and galactose-6-phosphate serve as substrates for G6PD isozymes, but soluble and particulate isozymes demonstrate different Km values for these substrates.

Significance to Cancer Research: (NCP Objective 6, Approach 3). These studies provide insight into the biochemical events related to growth of experimental hormonally dependent mammary carcinomas which mimic human cancers. Understanding of the mechanism of microsomal G6PD activity disappearance in the DBcAMP treated regressing tumor will elucidate the role of the cyclic AMP system in the control of abnormal growth.

Proposed Course of Research: To further investigate the molecular events of G6PD isozyme changes in relation to growth arrest and regression, future objectives include: (1) isolation and purification of MTW9 isozymes and determination of rates of synthesis and degradation; (2) determine whether DBcAMP action on G6PD activity is at transcriptional or translational level; (3) determine correlation of G6PD activity in growing and regressing tumors with hormone binding activity.

Keyword Descriptors: Glucose-6-Phosphate Dehydrogenase; isozymes; MTW9 rat mammary carcinomas.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 08214-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Construction of a Cage for Continuous Intravascular Infusion of Radioisotopes and Drugs in Small Animals.

Previous Serial No.: None

Principal Investigator: Pietro M. Gullino

Other Investigators: Flora Grantham, Donald Hill, Jess Rowland and Victor Frank

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS

Man Years:

Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objective: To devise a cage for the continuous intra-arterial or intravenous infusion of rodents with 4 major features: (1) The animal is free to move during infusion, (2) loss of infusate is avoided, (3) cleaning and decontamination is simple and effective, and (4) infusion can continue for weeks. There is no commercially available cage which satisfies our needs for both safety and reliability.

Major Findings: A cage meeting the requirements has been constructed. The government considered this cage as an invention and filed a patent application which has been accepted and is now being processed. U.S. Patent Application Serial No. 487032, July 10, 1974.

Significance to Cancer Research: A safe and efficient cage for continuous intravascular infusion of drugs and isotopes in small animals has been constructed.

Keyword Descriptors: Small animal, design and construction; intravascular infusion, experimental.

Publication: Grantham, F.H., Hill, D.M.,Rowland, J. and Gullino, P.M.: Cage for continuous intravascular infusion of isotopes and drugs in small animals. J. Natl. Cancer Inst. In press.

Project No. Z01 CBD 08215-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neovascularization: An Early Event in Neoplasms of the Human Mammary Gland

Previous Serial No.: None

Principal Investigator: Steven Brem

Other Investigator: Pietro M. Gullino

Cooperating Units: Animal holding facilities at Hazleton Laboratories; Bethesda Naval Hospital; Suburban Hospital

Man Years:

Total: 2 1/4
Professional: 1 1/4
Other: 1

Project Description:

Objectives: We are using neovascularization as a novel means of identifying neoplastic transformation at a very early stage in the human mammary gland. Recently, Folkman showed that tumors, early in their development, produce a vasoformative stimulus. In this laboratory, Gimbrone and Gullino showed that in the C3H-A^{vy} mouse, neovascularization was stimulated by malignant and premalignant lesions, but was absent in the normal breast tissue. Furthermore, the frequency of neovascularization induced by the premalignant lesion, the hyperplastic alveolar nodule (HAN), correlated with the statistical probability of neoplastic transformation.

Method: We have developed this work to a study of human breast tumors. In collaboration with Bethesda Naval Hospital and Suburban Hospital, we are currently assaying human breast tissue for angiogenesis. Biopsy specimens are transplanted immediately to the rabbit iris and cornea. Neovascularization is monitored with the slit-lamp stereomicroscope. The histology of the transplant is then correlated with its capacity to induce new blood vessels.

Significance to Cancer Research: Although the results are still preliminary and require a large sample of human breast tumors, we feel this will be a valuable approach to answer two questions: 1) Can angiogenesis be used as a marker to identify those lesions of the human breast that, like the mouse

HAN, have a high probability of neoplastic transformation? 2) In patients with breast cancer, is there a zone of neoplastic transformation beyond the actual confines of the tumor mass in the surrounding tissue?

Keyword Descriptors: Breast tumor; premalignant lesions; tumor angiogenesis; ocular transplants.

Honors and Awards: None

Publications:

1. Gimbrone, M.A., Jr., Gullino, P.M.: Neovascularization Induced by Intraocular Xenografts of Normal, Preneoplastic and Neoplastic Mouse Mammary Tissues, Fed. Proc. 33: 596, 1974.

Project No. Z01 CBD 08216-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In Vitro Synthesis of RNA in Isolated Nuclei

Previous Serial No.: None

Principal Investigator: Pradman K. Qasba

Other Investigators: Pon-Nyong Yi

Cooperating Units: None

Man Years:

Total: 1
Professional: 1
Others: 0

Project Description:

Objective: Previous studies showed that isolated nuclei from SV40 infected cells synthesized SV40 specific DNA but failed to initiate a new round of DNA replication. As the DNA synthesis requires the concomitant synthesis of RNA, we investigated various conditions influencing the RNA synthesis in isolated nuclei.

Methods Employed: Nuclei were isolated from AGMK cells using Triton X-100 and purified on Ficol gradients. Synthesis of RNA was carried out at different temperatures and salt conditions in the presence and absence of inhibitors. Synthesized RNA was characterized on sucrose gradients and polyacrylamide gels.

Major Findings:

1) Kinetics of RNA synthesis in the isolated nuclei from SV40 infected AGMK cells was shown to be temperature dependent. At 25°C net RNA synthesis was higher than at 37°C and continued for 30-40 minutes. At 37°C RNA synthesis continued only for 10-15 minutes and was then followed by degradation resulting in the loss of 30-40% of synthesized RNA.

2) Degradation of synthesized RNA was temperature dependent. Nuclei synthesizing RNA at 37°C when shifted to 25°C ceased to degrade synthesized RNA. In a reverse experiment when nuclei synthesizing RNA at 25°C were shifted to 37°C, there was first an increase in the rate of RNA synthesis followed by degradation.

3) Pulse chase experiments showed that there was no detectable degradation of synthesized RNA at 25°C. At 37°C degradation started 10-15 minutes after the initiation of synthesis.

4) Synthesis of RNA both at 25°C and 37°C was totally dependent on rGTP, rCTP, and on DNA template.

5) In the presence of low doses of actinomycin D 60% of the RNA synthesis was inhibited when nuclei were incubated for 5 min at 25°C or 37°C. At later periods of incubation at 25°C inhibition was only 30%. At 37°C in the presence of low doses of actinomycin D there was no detectable degradation of synthesized RNA. In the presence of high doses of α-amanitine, which normally inhibits all but r-RNA-synthesis,showed only 10-15% inhibition of RNA synthesis at 25°, whereas at 37° there was 40-50% inhibition of RNA synthesis and the degradation of RNA was not abolished.

6) Polyacrylamide gel electrophoresis of synthesized RNA showed that RNA synthesized at 25° or at 37° during the first 5 minutes mostly coelectrophoresed in the region of 18-28S. RNA at later periods of synthesis at 25° comigrated at the 4-5S as well as the 18-28S region. There was no observable change in the gel electrophoretical mobility of RNA synthesized between 20-60 min. RNA synthesized at 37°C showed by gel electrophoresis that the RNA comigrating in the 18 to 28S region decreases considerably during the 20 to 60 minute synthetic period.

Significance to Cancer Research: (NCP Objective 6, Approach 3). To develop means of detecting *in vitro* the differences in the RNA metabolism of normal and abnormal cells.

Proposed Course of Research. To identify which class of RNA is susceptible to this unique temperature dependent degradation.

Keyword Descriptors: RNA; *in vitro* synthesis; isolated nuclei; SV40; AGMK cells; temperature dependent; actinomycin D, α-aminitine.

Honors and Awards: None

Publications:

1. Qasba, P.K.: Synthesis of SV40 DNA in Isolated Nuclei. Proc. Nat. Acad. Sci., USA, 71: 1045, 1974.

2. Qasba, P.K.: Synthesis of SV40 DNA in Isolated Nuclei. Biochem. Biophys. Res. Comm. 60: 1338, 1974.

3. Qasba, P.K., Yi, P.N. and Finlason, B.: *In Vitro* Synthesis of RNA in Isolated Nuclei. Fed. Proc. In Press.

4. Aposhian, H.V., Thayer, R.E. and Qasba, P.K.: Formation of Nucleoprotein Complexes Between Polyoma Empty Capsids and DNA. Journal of Virology 15: 654-653, 1975.

Project No. Z01 CBD 08217-01 LPP
1. Laboratory of Pathophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: α-Lactalbumin, Mammary Carcinoma Cells, Synthesis.

Previous Serial No.: None

Principal Investigator: Pradman K. Qasba

Other Investigator: Pietro M. Gullino

Cooperating Unit: None

Man Years:

Total: 1 5/8
Professional: 1 1/8
Other: 1/2

Project Description:

Objectives: To search for a mammary carcinoma cell capable of synthesizing α-lactalbumin - a mammary tissue specific protein - and to study its hormone dependent gene expression during growth and regression of the mammary tumor.

Methods Employed:

1) Lactose synthetase assay: α-lactalbumin, which is a part of lactose synthetase, is assayed by measuring the conversion of UDP-[^{14}C] galactose into lactose in the presence and absence of glucose. The non-ionic-radioactive products (galactose and lactose) are separated on Dowex AG 1-X8 - an anion exchanger - column.

2) Human mammary tumor cell lines MDA-MB 231, MDA-MB 157, are grown in the presence of insulin, hydrocortisone and bovine prolactine.

3) Rat mammary tumors R3230Ac, MTW9 and 13762 MAT are passed on in appropriate strains of rats.

Major Findings: Rat mammary tumors R3230Ac and MTW9 and 13762 MAT a and b show nearly 10-fold higher levels of lactose synthetase than virgin mammary gland, but the levels are nearly one-tenth of 4-10 day lactating mammary gland. 13762 MAT c show no detectable lactose synthetase activity. Human mammary tumor cell lines MDA-MB 231 and MDA-MB 157 have also shown higher levels of lactose synthetase than WI-38 - a human fibroblast cell line. All the tumor cell lines have shown a very high activity of UDP-galactose hydrolase.

Proposed Course of Research: To identify human and rat α-lactalbumin directly by radioimmunoassay.

Significance to Cancer Research: By developing a very sensitive method detecting α-lactalbumin one could characterize the functional differentiation of neoplastic mammary cells and correlate it with hormone dependency.

Keyword Descriptors: α-lactalbumin; synthesis; mammary tissue specific protein; hormone dependent; mammary carcinoma; R3230Ac (rat); MTW9 (rat); 13762 MAT (rat); MDA-MB 231 (human); MDA-MB 157 (human); radioimmunoassay.

Honors and Awards: None

Publications: None

LABORATORY OF THEORETICAL BIOLOGY

SUMMARY

July 1, 1974 - June 30, 1975

Summary Report

The activities of the Laboratory of Theoretical Biology (LTB) fall into several areas: the biophysics of membranes, developmental biology, molecular configurations, kinetic metabolic systems and mathematical and computational methodology and theory related to modeling. Most of the work is theoretical, although some experiments are also carried out in the laboratory. A number of the modeling activities are in collaboration with experimental groups both at NIH and elsewhere.

The work on membranes includes several areas of interest, mostly related to lipid bilayer systems (Blumenthal). Studies have been carried out on conductance changes produced by "Excitability Inducing Material" (EIM) which provide insights into the gating of ion conducting pores. Ionophoric properties such as voltage dependent conductance, conductance fluctuations, ion selectivity and pH dependence were investigated for a number of substances: antibiotic released from A. Cloacae, peptide from eel electroplax membranes, dopamine-β-hydoxylase, hemocyanin, valinomycin, gramicidin. Since some substances act both as ionophores and as B-cell mitogens, a relationship between the two is suspected. Preliminary work is also in progress, using the lipid bilayer membranes, to study the first phases of lymphocyte mediated cytolysis. Observed conductance changes produced by lymphocyte-membrane interaction in the presence and absence of antibodies suggest that cytolysis of antibody coated tanget cells is a direct consequence of the breakdown of the permeability barrier of the target cell.

Another problem related to membranes is the modeling of the mechanism related to adenylate cyclase activity in membrane preparation (Rendell, Berman). This is a collaborative study with Dr. Rodbell's group in NIAMDD. A previous model has been further refined to include two activated and one inactivated state of the enzyme, and a catalytic site that interacts with the substrate (ATP) and an inhibitor (HATP). This model has been tested and found compatible with the data, and equilibrium constants for these states have been determined. Originally developed for liver cell membranes, the model is also being applied to interpret adrenal cell cyclase activity. Transient behavior of the model is also being tested.

Analysis of mechanisms underlying determinations and transdeterminations in Drosophila (Dr. Kauffman) is an experimental effort to verify (and to extend) a theory on control circuits in developmental biology. According to Kauffman's theory, the determination of wings, legs, antennas, etc., from imaginal discs in Drosophila are controlled by a circuit composed of at least four bistable states (for example, leg = 1010, wing = 1111, etc.). A transdetermination is a chance switch of one or more circuits from one state to another. Efforts are under way to fuse cells from different imaginal discs to determine the dominance of the various circuits. Stocks for marking clones by somatic recombinations have been constructed. Attempts to characterize "epigenetic code" by mutants which switch circuit states leading to coordinated alterations in transdetermination frequencies are also under way.

A project new to this laboratory is the study of polypeptide configurational kinetics (Jernigan, Szu). The relaxation times of polypeptides are calculated with relation to their transitions from helical to random-coil configurations. Factors such as molecular size, equilibrium parameters, and friction have been included in the model. The calculations permit the interpretation of relaxation data in terms of molecular step rates.

The development of theory to achieve greater insights into oscillatory phenomena is in progress (Trabert). The Bendixon theory, applicable to two dimensional oscillators has been extended to three dimensions. Using this theory, one can evaluate proposed dynamic models as to whether they may exhibit oscillatory behavior.

Several metabolic systems are being actively modeled by this group, usually in collaboration with one or more experimental groups at NIH or elsewhere. The modeling of metabolic systems has now reached a high level of complexity and sophistication and on-hand experience in dealing with large systems provides new insights into the theory and methodology of modeling as related to large systems. This activity also serves as a pilot project in establishing model-systems for several important metabolic systems in man.

The lipoprotein system (Berman, Hall, & Malmendier) is modeled in collaboration with Dr. Levy's group (NHLI) and Dr. Malmendier (University of Brussels). The model previously developed for apo-B proteins using an endogenous label (Dr. Fisher) has been extended to exogenously labeled apo-B and apo-C protein studies carried out by Dr. Levy's group. A more complete and extensive model has evolved now that covers apo-B and apo-C kinetics in very low density (VLDL), intermediate density (IDL), low density (LDL) and high density (HDL) lipoproteins. These studies have now been complemented by endogenously labeled lipoproteins kinetics (Malmendier) involving simultaneous FFA and leucine labeling of the triglyceride and apoprotein moieties. These new studies tend to confirm our previous model, and new features with respect to recycling and other pathways are emerging. Kinetic studies of HDL apo-A lipoproteins (Blum) have also been carried out and integration of this model with one previously obtained for HDL apo-C is in progress.

The modeling of the glucose-insulin system (Berman, Goebel, McGuire, Andres, and Hetenyi) involves two phases. In collaboration with Dr. Andres' group, NIA, the insulin secretion modeling and the study of the control mechanism of insulin on glucose utilization is continuing. A refined model that permits a better understanding of peripheral (muscle) glucose utilization and that predicts the relations between arterial, venous and "hot hand" response curves for glucose has been worked out. Analysis is also in progress of insulin, proinsulin and c-peptide kinetic curves in the rat (Rubinstein, Chicago), in the hope of getting a better insight into the secretion and degradation mechanisms of insulin. Detailed modeling is also being carried out on the interconversions of glucose, lactate, alanine and glycerol - both in man and dogs. Some theoretical considerations related to these, involving the Krebs cycle, are also being formalized.

In connection with thyroid hormone and iodine metabolism, a model has been formulated to account for the kinetics of iodide (I^-), thyroxine (T_4) and triiodothyronine (T_3) in the mother sheep and its fetus (R. McGuire, Berman). The model suggests that there is no T_3 utilization in the fetus and that T_3 transport across the placenta greatly favors the mother. The amniotic fluid emerges as an important store of both T_3 and T_4 hormones. A high T_3 gradient between fetal plasma and amniotic fluid was predicted by the model and subsequently confirmed experimentally.

The modeling of stomach emptying and secretion in the monkey and in humans has continued (Dubois, NIAMDD).

Work is progressing on the SAAM computer program and supporting activities (Berman, Weiss, Chu and McGuire). New extensions have been added to SAAM (Weiss) and new numerical analysis procedures have been introduced to permit the modeling of more complex systems (Chu). The conversational SAAM capability implemented on the PDP-10 computer at NIH (McGuire) has been further improved. The remote terminal software has been improved in reliability and new capabilities have been added for communication with two computers (Bidinger).

Project No. Z01 CBD 08300-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: SAAM Computer System

Previous Serial Number: NCI-8300

Principal Investiagtor: Mones Berman

Other Investigators: Marjory F. Weiss
Robert McGuire
Sherwood Chu

Man Years:

Total:	1.5
Professional:	1.5
Others:	0

Project Description:

Objectives: To develop a general purpose computer program for modeling biokinetic systems that may readily be used by investigators not sophisticated in mathematics or programming. Initiated in 1959, the program continues to be expanded and revised as new features are added.

Major Findings: A major revision of the structure of the program was undertaken and completed to permit additional features to be added to the program. Greater efficiency in operation and greater ease of use were additional results of this revision. New features have permitted the solution of some problems not previously within the scope of the SAAM program (Weiss, Chu).

The conversational version of SAAM implemented on the PDP-10 computer at NIH has been further improved (R. McGuire).

Significance for Cancer Research: The SAAM computer program has been a most essential tool in the modeling of various systems described elsewhere in this Laboratory report. Its wide use in other centers in the United States and elsewhere is further evidence of its value in biomedical research. It is consistent with Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: Broadening of the scope of the program, its use as a conversational tool and refinement of mathematical and statistical methods will continue.

Project No. Z01 CBD 08300-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Keyword Descriptors: Computer program, modeling, kinetic systems, mathematics, SAAM, conversational.

Publications:

Berman, M., and Weiss, M.F.: SAAM Manual, Revised 1974 (about 200 pages).

Project No. Z01 CBD 08301-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modeling Theory and Applications

Previous Serial Number: NCI-08301

Principal Investigator: Mones Berman

Other Investigators:
Robert Goebel
Paul Van Eerdewegh
Elizabeth A. McGuire (NICHD)
Reubin Andres (NICHD)
Andre Dubois (NIAMDD)
Margaret Hays (VA Central Office, Wash., D.C.)
Geza Hetenyi (Univ. of Ottawa, Canada)

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives: Analysis of data on metabolic systems, and further development of modeling theory and techniques through the applications of the SAAM computer program.

Major Findings: The glucose-insulin model has been further developed. A model was formulated to explain the differences in the glucose kinetics between arterial and venous samplings. This model also permits the interpolation to "hot hand" data (E.A. McGuire). The glucose model has also been further refined to include interconversion pathways between glucose and lactate and alanine in man (Goebel). Similar development for the dog defines glycerol to glucose and alanine to glucose conversions (Hetenyi).

The modeling of the stomach emptying and secretion processes in man and monkeys have continued (Dubois).

The development of qualitative criteria to describe models and data and to test compatibility between the two has continued (Van Eerdewegh).

Project No. Z01 CBD 08301-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance to Cancer Research: The methodology of modeling is most essential for studying the behavior of complex systems for both normal and abnormal states. This most closely fits into Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: This is a continuing process for the development of theory for and the application of modeling techniques.

Keyword Descriptors: Modeling, SAAM, glucose kinetics

Publications:

Insel, P.A., Kramer, K.J., Sherwin, R.S., Liljenquist, J.E., Tobin, J.D., Andres, R., and Berman, M.: Modeling the Insulin-Glucose System in Man. Federation Proceedings, July, 1974.

Leme, P.R., Creaven, P.J., Allen, L.M., and Berman, M.: A Kinetic Model for the Disposition and Metabolism of Moderate and High Dose Methotrexate in Man. Cancer Chemotherapy Report. In press.

Project No. Z01 CBD 08302-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Computational Mathematics

Previous Serial Number: 8302-NCI

Principal Investigator: Sherwood C. Chu

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To evaluate, develop and implement mathematical techniques used in the modeling and simulation of biological systems. This entails the development of efficient numerical procedures and their computational implementation.

Methods Employed: The primary mathematical tools needed to evaluate or develop an algorithm are from the theories of differential equations, numerical analysis, and linear algebra. Computer programming provides the means for implementation.

Major Findings: The numerical procedures and algorithms used in the solution of initial value problems for general systems of ordinary differential equations have been improved with regard to accuracy and efficiency.

A major effort was devoted to the problem of finding asymptotic values of solutions of non-linear differential equations. Among other considerations, this requires the determination of the solvability of the problem for any given system, the development of an algorithm for the solution of systems of non-linear algebraic equations, and the development of numerical quadrature techniques for integration over infinite time intervals.

In conjunction with Mrs. M.F. Weiss, the above are being implemented and incorporated into the SAAM program for routine usage.

Project No. Z01 CBD 08302-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance for Cancer Research: Sound computational techniques facilitate and permit the mathematical analysis of complex biological systems; it, thus, is consistent with Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: The analysis of the various mathematical aspects of biological modeling will be continued. An effort will be made to broaden the applicability of existing algorithms to more general systems as well as making them more computationally efficient.

Keyword Descriptors: Numerical analysis, ordinary differential equations, algebraic equations, asymptotic values.

Publications:

Chu, S.C., and Berman, M.: An Exponential Method for the Solution of Systems of Ordinary Differential Equations. Communications of the ACM. 17: 699-702, 1974.

Project No. Z01 CBD 08303-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ion Transport Through Modified Lipid Bilayer Membranes

Previous Serial Number: NCI 8303

Principal Investigator: Robert Blumenthal

Cooperating Units: Immunology Branch, NCI (NCI Z01 CBD 5018(c)-04 I)
Laboratory of Microbiology and Immunology, NIDR
Reproduction Research Branch, NICHD

Man Years:

Total:	0.9
Professional:	0.9
Others:	0

Project Description:

Objectives: To study the physical mechanisms for ion transport in reconstituted membranes. To develop the lipid bilayer membrane as an assay for ion-conductance inducing materials isolated from natural cell membranes. To study the interaction of lymphocytes with lipid bilayer membranes as a model for lymphocyte mediated lysis of target cells.

Methods Employed: The bilayer membranes are formed from natural membrane extracts, oxidized cholesterol or pure lipids in an aperture between two electrolyte solutions. The electrical properties of the membranes are measured before and after application of an activating factor. In the lymphocyte experiments DNP-phosphatidyl ethanolamine was incorporated into a horizontal lipid bilayer, the settling of the lymphocytes on the lipid bilayer was observed through a phase contrast microscope and the electrical conductance was measured.

Major Findings:(1)The ion conducting properties of dissociated and associated hemocyanin, which was found to produce conductance changes in lipid bilayer membranes, were studied in relationship with their mitogenic activity on B-cell lymphocytes. The current-voltage curves, conductance concentration relationship and discrete current fluctuations show marked differences between dissociated and associated hemocyanins. The studies are interpreted in terms of channel mechanism for ion conductance of associated and a "defect" mechanism for the ion conductance of dissociated hemocyanin. With respect to B-cell mitogenesis we found that both excitability inducing material and hemocyanin are potent B-cell mitogens. Other ion conductance mediating compounds such as valinomycin and gramicidin do not trigger mitogenesis, whereas other mitogens (endotoxins) do not mediate ion conductance in lipid bilayer membranes.

2) Purified dopamine-β-hydroxylase (DBH) at a concentration of 0.4 μgr/ml induces conductance change in lipid bilayer membranes. Conductance-DBH concentration curves yield a slope of one on a logarithmic scale, the conductance—potassium chloride concentration curve shows a threshold effect; diffusion potential measurements yields a cation transference number of 0.8 and low level discrete current fluctuations indicate a channel mechanism. The data suggest that DBH may be linked with an ion-conducting process *in vivo*.

3) Lymphocytes induce a conductance increase of several orders of magnitude in antibody coated lipid bilayers, without resulting in breakage. Neither antibody alone nor lymphocytes alone show this effect. The conductance increase is dependent on a membrane potential which is positive on the lymphocyte side with respect to the other side, as is the case with a natural target cell membrane. Small discrete conductance increases are seen in some experiments, leading to the suggestion that lymphocytes are creating ion-conducting channels in the bilayer.

Significance for Cancer Research: The lipid bilayer membrane has been proven to be a sensitive probe for the molecular properties of ion-conducting channels in membranes. I have extended my initial efforts to study the permeability function of natural membranes in a well-defined and controlled system. With respect to cancer the regulatory functions of ion permeability include cell division, pseudopod formation and gating of cell junctions. This work supports Objective 3, Approach 5 of the National Cancer Plan.

Proposed Course of the Project: We are attempting to establish a relationship between ion-conducting activity and B-cell mitogenesis. In the case of the endotoxins we plan to study their interaction with the membrane surface by looking at the effect of the electrical double layer produced by the endotoxin-lipid bilayer surface on valinomycin mediated conductance.

In the case of dopamine-β-hydroxylase we plan to characterize the conductance mediator further and to look at effects of catecholamines on the conductance.

Lymphocyte studies are continuing to define the nature of the induced conductance increase by studying selectivity, current fluctuations, voltage-current relationships, dependence of conductance on the dose of antigen, antibody and lymphocyte. We plan to study antigen antibody binding on the bilayer by using fluorescent labeled antibody and to observe its distribution on the bilayer in the presence and absence of lymphocytes. We also intend to extend the studies to T-cell lymphocytes.

Project No. Z01 CBD 08303-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Keyword Descriptors: Membranes, bilayers, ion channels, ionophores, lymphocytes, hemocyanin, dopamine-β-hydroxylase, cytolysis, mitogenesis, conductance.

Honors and Awards: Invited to organize and chair a panel discussion on "The Lipid Bilayer Membrane As An Assay For Ion Conductance Inducing Materials Isolated From Biological Membranes" held at the "International Conference On Carriers and Channels in Biological Systems" by the New York Academy of Sciences, February 24-27, 1975 in New York, N.Y.

Publications:

Ehrenstein, G., Blumenthal, R., Latorre, R., and Lecar, H.: The Kinetics of the Opening and Closing of Individual EIM Channels in a Lipid Bilayer. J. Gen. Physiol. 63: 707, 1974.

Shamoo, A.E., Myers, M.M., Blumenthal, R., and Albers, R.W.: Ionophoric Material Derived from Eel Membrane Preparations, I. Chemical Characterization. J. Membrane Biol. 19: 129, 1974.

Blumenthal, R., and Shamoo, A.E.: Ionophoric Material Derived from Eel Membrane Preparations, II. Electrical Characteristics. J. Membrane Biol. 19: 141, 1974.

Blumenthal, R., and Pollard, H.B.: Incorporation of Dopamine-β-Hydroxylase into a Lipid Bilayer Membrane. Biophys. J. 15:13a, 1975.

Project No. Z01 CBD 08304-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Dynamic Membrane Transport Models

Previous Serial Number: NCI-8304

Principal Investigator: Dr. Robert Blumenthal

Man Years:

Total:	0.1
Professional:	0.1
Others:	0

Project Description:

Objectives: To develop models describing transformations of levels of cellular activity induced by transient environmental stimuli at the membrane level.

Methods Employed: Expressions are derived for the transient and steady state concentration levels of metabolite on the membrane surface. Simulations are carried out making use of the PDP-10 of the central NIH computer facility.

Significance for Cancer Research: The models are applied to 1) transitions of respiratory states in mitochondria, of uptake of metabolites in transformed cells and of levels of inducer in differentiating cells; 2) sustained oscillatory behavior of cells as a basis for development; 3) pattern formation of molecules on the membrane surface in the study of the dynamic architecture of membranes. This project most closely supports Objective 4, Approach 4 of the National Cancer Plan by developing mathematical models of the cancerous process.

Proposed Course of Study: The models will be further developed in relation to biological data on dynamic membrane phenomena.

Keyword Descriptors: Membranes, transport, models, oscillations, morphogenesis.

Publications:

Blumenthal, R.: Thermodynamic and Kinetic Analysis of Functional Linkage. In F.O. Schmitt, D.M. Schneider and D.M. Crothers (ed.): Transmembrane Transport in Functional Linkage in Biomolecular Systems. New York, N.Y., Raven Press, 1975, pp. 149-158.

Blumenthal, R.: Instabilities, Oscillations and Chemical Waves in an Oligomeric Model for Membrane Transport. J. Theoret. Biol. 49: 219-239, 1975.

Project No. Z01 CBD 08305-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Remote Communications Computer System

Previous Serial Number: 8305-NCI

Principal Investigator: Peter A. Bidinger

Other Investigator: Florence K. Millar

Cooperating Unit: DOD Computer Institute
Washington Navy Yard
Washington, D.C. 20374

Man Years:

Total:	0.25
Professional:	0.25
Others:	0

Project Description:

Objectives: To continue the development of a remote terminal computer operating system which will interface with various other computers and utilize various on-line input-output devices, to meet the needs of the Laboratory of Theoretical Biology.

Major Findings: The Univac 1004 emulator has been further expanded to include a local teletype and asynchronous lines allowing connection to computers in timesharing demand mode with 1200 band communication rate as an option. This addition makes full use of the various input-output devices of the PDP-11/20 computer, provides a back-up printer for the system, and further permits the transfer of information between two separate external computer systems, transfer being controlled locally at the PDP-11/20.

Key Word Descriptors: Remote terminal computer system, teleprocessing, telecommunication, Univac 1004 emulator, computer interface, PDP-11.

Project No. Z01 CBD 08306-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Kinetic Modeling of Human Plasma Lipoprotein Metabolism

Previous Serial Number: NCI-8306

Principal Investigator: Mones Berman

Other Investigators: Marshall Hall, III
Claude Malmendier

Cooperating Units: Molecular Disease Branch, NHLI
(Drs. R. Levy and C. Blum)
University of Brussels, Belgium
(Dr. C. Malmendier)

Man Years:

Total: 1.2
Professional: 1.2
Other: 0

Project Description:

Objectives: To develop a qualitative and quantitative understanding of lipoprotein metabolism in man and to identify abnormalities through the modeling of lipoprotein kinetics. Because of the need for diverse, extensive data on a variety of patients, collaboration with several experimental groups was established.

Methods Employed: Mathematical modeling is the tool used for the integration and analysis of the data. This is performed with the help of the SAAM computer modeling program.

Major Findings: Synthesis of a previously developed model for apo-B kinetics with the more recent model for apo-C was completed on a series of 16 studies involving normals and abnormal patients. A more confident model that accounts for the metabolism of very low density (VLDL), intermediate density (IDL), low density (LDL) and high density (HDL), apolipoproteins emerged. New data on the kinetics of apo-A in HDL have been collected by the NHLI group and a model for this is now being developed. New data have also been collected by the Brussels group (Malmendier) on endogenous incorporation of leucine and FFA into lipoproteins and the kinetics of endogenously labeled lipoproteins. The models developed for exogenously labeled lipoproteins is being tested and modified to satisfy the new data. Some new metabolic patterns seem to have emerged but it is still too early to claim these with confidence.

Project No. Z01 CBD 08306-03 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance for Cancer Research: Modeling becomes important in trying to isolate the abnormality responsible in hyperlipemics, given a very complicated system with complicated interactions. In the long run, it is hoped that by isolating the abnormality, treatment can be geared to the abnormal site identified. Clinically, this is relevant to atherosclerosis, cardiovascular disease, and other metabolic disorders. Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: The results obtained with protein kinetics will be further integrated with experiments involving other lipoprotein moieties, such as the triglycerides. Some work on cholesterol will also be initiated.

Keyword Descriptors: Lipoprotein metabolism in man, kinetics, hyperlipoproteinemia, model, VLDL, IDL, LDL, HDL, lipoprotein.

Publications:

Malmendier, C.L., Delcroix, C., and Berman, M.: Interrelations in the Oxidative Metabolism of Free Fatty Acids, Glucose and Glycerol in Normal and Hyperlipemic Patients. J. Clin. Invest. 54: 461-476, 1975.

Phair, R.D., Hammond, M.G., Bowden, J.A., Fried, M., Fisher, W.R., and Berman, M.: A Preliminary Model for the Metabolism of Human Lipoproteins in Type IV Hyperlipoproteinemia. Federation of American Societies for Experimental Biology Proceedings, 1975. In press.

Insel, P.A., Liljenquist, J.E., Tobin, J.D., Sherwin, R.S., Watkins, P., Andres, R., and Berman, M.: Insulin Control of Glucose Metabolism in Man: A New Kinetic Analysis. J. Clin. Invest. In press.

Project No. Z01 CBD 08312-02 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Computer Applications: Programming and Data Processing

Previous Serial Number: 8312-NCI

Principal Investigator: Florence K. Millar

Man Years:

Total:	0.75
Professional:	0.75
Others:	0

Project Description:

Objectives: To facilitate the investigation of biomedical research problems through the application of computer programming and computer data processing.

Methods Employed: Use is made of the PDP-11/20 computer of this laboratory, the National Bureau of Standards Univac 1108 computer, and the central NIH computer facility. The projects reported may be collaborative efforts with investigators in other units, as shown below.

Major Findings:

Computer Applications:

(1) Analysis of feeding patterns in rats (Dr. S.D. Morrison, C/LPHY). Punched paper tape output, produced as rats feed from an eatometer, gives, after appropriate computer corrections, direct measurement of feeding intensity. Readings are checked by computer for significance above background noise and for instrument error and are then combined to give actual meal lengths and inter-meal intervals. The dependency of the feeding patterns thus obtained upon the definition of various meal parameters has been examined.

(2) Size distributions of thyroid follicles, cells and cell components. (Dr. J.P. Herveg, C/LPHY). Two-dimensional distributions of cell size parameters (e.g., major and minor axes) have been analyzed by the method of Wicksell (Biometrika 17:84, 1925) using the program of Baudhuin (Methods in Enzymology 32:3, 1974) to calculate the actual distributions in three dimensions of these tissue components.

Project No. Z01 CBD 08312-02 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

(3) Processing and plotting of data from liquid scintillation spectrophotometers; comparison of isoaccepting species of tRNA isolated from normal and malignant cells. (Dr. J.F. Mushinski, C/LBGY)

(4) Analysis of enzyme kinetics; studies on UMP-CMP kinase and uridine-cytidine kinase. (Dr. E.P. Anderson, Mr. A.S. Liacouras, C/LPHY; Dr. T.Q. Garvey, III, Mass. General Hospital.) Use is made of programs developed by Cleland (Nature 198:463, 1963) to handle the reaction equations.

(5) Plotting of data from gastrointestinal studies. (Dr. A. Dubois, A/DDB) The CalComp plotter on line to the PDP-11 computer is used for plotting data obtained in studies of factors involved in gastrointestinal activity. Data, processed and punched on paper tape at another computer terminal, are read into the PDP-11. The program allows for choice of variables to be plotted, labelling and design of the plot, and scaling of the data.

Modeling and Simulation with the SAAM Computer Program:

(1) Calcium uptake by the thyroid. (Dr. A. Haeberli, C/LPHY) Both a two- and a three-compartment system were used to model the data.

(2) Analysis of flow microfluorometry data; analyses of DNA content of individual cells (Dr. H. Metzger, A/A&R). A sum of Gaussian distributions linearly related was used to model the data.

Significance to Biomedical Research and the Program of the Institute: Computer programming applications contribute to the investigation of research problems by providing (1) accurate and efficient methods for handling laborious calculations of data and visualizing results, (2) the ability to store and retrieve large amounts of data, and (3) opportunities for testing mathematical models based on experimental results. This project most closely supports Objective 4, Approach 4 of the "National Cancer Plan" by providing methods of developing mathematical models of the cancerous process.

Proposed Course of Study: Programs will continue to be developed and computational techniques to be applied in response to computer-related needs of the scientific staff.

Publication: Liacouras, A.S., Garvey, T.Q., III, Millar, F.K. and Anderson, E.P.: Uridine-cytidine kinase. II. Kinetic studies and reaction mechanism. Arch. Biochem. Biophys., 1975, in press.

Key Word Descriptors: Computer applications, computer programming, software, mathematical applications, biomedical research.

Project No. Z01 CBD 08313-02 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Analysis of Mechanisms Underlying Determination and Transdetermination in Drosophila

Previous Serial Number: NCI-8313

Principal Investigator: Stuart Kauffman

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: Determination and transdetermination in imaginal discs of Drosophila must be underlain by some form of control system having sharply alternate self maintained states. If many such subsystems or circuits are involved, the combinations of their states may be characterized as an epigenetic code. The major objective is to uncover the logical structure of that code. Recently, I suggested determination is due to at least four circuits each with two alternate states. Thus leg ≡ 1010, antenna ≡ 1100, wing ≡ 1111, where 1 is the more stable state of each circuit. To test this model, I hope to achieve somatic cell fusion of disc cells, then test the determined state of the fused cell. If the more stable "1" state wins any competition, leg x wing = 1010 x 1111 yields 1111 or wing, while haltere fused with antenna (0111 x 1100 → 1111) should yield wing.

Methods Employed: Dr. H. Schneiderman has supplied three complementing temperature sensitive cell lethal mutants of Drosophila. Each carries a second visible cuticle marker. I am disaggregating and coreaggregating 3rd instar imaginal discs of two T.S. strains, treating with various chemical and viral agents to induce fusion, implanting the aggregate in 3rd instar larvae to induce the metamorphosis of the reaggregate with the host, and hoping to find fused cells. Such cells should be characterized by being wild type for cuticle markers, and wild type for the complementing T.S. mutants, hence I should be able to select for rare fusion events at the nonpermissive temperature and recognize fused cells. In addition, I am searching among homeotic mutants, to find those which switch a determination circuit from one to another state. This requires use of somatic recombination to mark clones, and experiments to distinguish whether homeotic conversion, say, of antenna to leg, is due to an alteration in determination itself, or other processes.

Project No. Z01 CBD 08313-02 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Major Findings: Feeding Brome deoxyuridine (BUDR) to larvae reduces the penetrance and expressivity of the temperature sensitive aristapedia mutant, which converts distal antenna to distal leg. Three stocks for marking clones by somatic recombination have now been constructed.

Significance for Cancer Research: Imagine that one knew for each pair to be fused (e.g., wing x leg → ?) what it yielded. The matrix listing all possible pairs and the result will be a powerful clue to the logical structure of the decision processes underlying determination and transdetermination. I take it that illuminating that structure and its material makeup constitute the central problem of developmental genetics. Objective 3, Approach 1 of the National Cancer Plan applies.

Proposed Course: Try various agents, lysolecithin, concanavalin A, sendai virus, in attempts to obtain fusion. Attempt to characterize "epigenetic code" by mutants which switch circuit states, leading to coordinated alterations in transdetermination frequencies.

Keyword Descriptors: Determination, transdetermination, imaginal discs, Drosophila, control system having sharply alternate self-maintained states epigenetic code.

Project No. Z01 CBD 08314-02 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modeling of Adenylate Cyclase

Previous Serial Number: NCI-8314

Principal Investigator: Marc Rendell and Mones Berman

Other Investigator: Martin Rodbell

Cooperating Units: Laboratory of Nutrition and Endocrinology
NIAMDD

Man Years:

Total	2.5
Professional:	2.5
Others:	0

Project Description:

Objectives: The kinetics of adenyl cyclase have been investigated in the hepatocyte, the adrenal, and the fat cell. The mechanism of catalysis has been described as well as the mode of regulation by nucleotides and hormones. Further objectives include the investigation of changes in cyclase behavior in malignancy using kinetic analysis.

Methods Employed: Sophisticated techniques of simulation and analysis are used to construct experiments yielding optimal kinetic information about the enzyme.

Major Findings: Adenyl cyclase has now been shown to possess three intrinsic states of catalytic activity showing marked differences in several parameters of catalysis. Guanine nucleotides and hormones of various types have been shown to act on adenyl cyclase by modulating the distribution among these states.

Significance to Biomedical Research and the Program of the Institute: This work comes under the National Cancer Plan 4.4. If cell transformation does indeed proceed via changes in adenyl cyclase catalysis, the kinetic framework currently developed will be of great importance in following the sequence of changes leading to transformation.

Proposed Course: The kinetics of adenyl cyclase in malignancy may well be the next step in this work.

Project No. Z01 CBD 08314-02 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Keyword Descriptors: Glucagon, adenyl cyclase, ACTH, Computer simulation, Enzyme kinetics.

Publications:

Rodbell, M., Lin, M.C., Salomon, Y., Londos, C., Harwood, J.P., Martin, B.R., Rendell, M., and Berman, M.: The Role of Adenine and Guanine Nucleotides in the Activity and Response of Adenylate Cyclase Systems to Hormones: Evidence for Multi-Site Transition States. Acta Endocrinologica. 77: 11-37, 1974.

Salomon, Y., Lin, M.C., Londos, C., Rendell, M., and Rodbell, M.: The Hepatic Adenylate Cyclase System. I: Evidence for Transition States and Structural Requirements for Guanine Nucleotide Activation. J. of Biol. Chem. In Press.

Lin, M.C., Salomon, Y., Rendell, M., and Rodbell, M.: The Hepatic Adenylate Cyclase System. II: Substrate Binding and Utilization and the Effects of Magnesium Ion and pH. J. of Biol. Chem. In Press.

Rendell, M., Salomon, Y., Lin, M.C., Rodbell, M., and Berman, M.: The Hepatic Adenylate Cyclase System. III: A Mathematical Model for the Steady State Kinetics of Catalysis and Nucleotide Regulation. J. of Biol. Chem. In Press.

Rodbell, M., Lin, Salomon, Y., Londos, C., Harwood, J.P., Martin, B.R., Rendell, M., and Berman, M.: The Role of Adenine and Guanine Nucleotides in the Activity and Response of Adenylate Cyclase Systems to Hormones: Evidence for Multi-Site Transition States. In Greengard and Robison (Eds.): Advances in Cyclic Nucleotide Research, Vol. 5, New York, Raven Press. (In press).

Project No. Z01 CBD 08315-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Analytical Studies of Stability and Dynamical Behavior of Models Simulating Biological Systems

Previous Serial Number: NHLI-298

Principal Investigator: Kenneth K. Trabert

Cooperating Units: Dr. Donald Tschudy
Metabolism Branch, NCI

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: Mathematical models which simulate general biochemical kinetic reactions were studied by analytical means to determine regions of relative stability.

Methods Employed: Theorems were developed to determine whether the stability was a fixed point or a moving point kind. In addition, a theorem was produced which can be used to determine which models will or will not show oscillatory behavior in three dimensional systems. The technique could not be generalized to four dimensions.

Major Findings: These theorems are useful in determining whether one has equilibrium points or regions of oscillation in biochemical kinetic models or other systems which may be modeled with differential equations. If there is a diseased state which is rhythmic and a normal state which is not, then these theorems are useful in establishing differences in the two model systems. In particular, these techniques were applied to a kinetic model for heme metabolism in acute intermittent porphyria and the normal, ruling out the possibility of oscillatory phenomena for a particular model used to represent the normal state. Stability analysis of models simulating biological systems will be continued but the man hours of time devoted to this project will be reduced.

Significance for Cancer Research: This project most closely supports Objective 4, Approach 4 of the National Cancer Plan by developing mathematical models of the cancerous process.

Keyword Descriptors: Mathematical models, biochemical kinetic reactions, heme metabolism in acute intermittent porphyria and the normal, regions of relative stability, equilibrium points, kinetic model, biochemical kinetic model.

Project No. Z01 CBD 08316-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Kinetic Modeling of Thyroid Function in Man and Animals

Previous Serial Number: None

Principal Investigators: Mones Berman and Robert McGuire

Other Investigators: Bruce Belshaw (Cornell Medical Center, N.Y.)
Marguerite Hays (V.A. Central Office, Wash., D.C.)

Man Years:

Total:	0.7
Professional:	0.7
Other:	0

Project Description:

Objectives: To develop a quantitative understanding of thyroid hormone synthesis, distribution and catabolism in man and other animals; to study the changes occurring during normal development and during disease states; and to study the effects of pharmacologic agents in the thyroid system.

Methods Employed: Integration and analysis of collaborative studies and literature information within a comprehensive mathematical model. The model is developed using the SAAM computer modeling system.

Major Findings: Models for lithium distribution in man, rat and dog were developed to gain a further understanding of the lithium effects on thyroid hormone release and peripheral metabolism previously observed.

A comprehensive model of iodide (I^-), thyroxine (T_4) and triiodothyronine (T_3) transport in maternal and fetal sheep was developed. The model predicted significant concentrations of each material in amniotic fluid, which were confirmed by subsequent experiments. Other findings included: 1) very little T_3 synthesis or degradation within the fetus; 2) negligible T_4 exchange between mother and fetus. Development of models from thyrotropin (TSH), and thyrotropin releasing hormone (TRH) were initiated in order to investigate the control of thyroid hormone release.

The iodine kinetics model developed for the beagle (Belshaw, 1974) is now being applied to a series of studies involving variations in dietary iodine intakes. Changes in parameter values due to this effect have been observed. Further refinement of the model was also achieved with the new studies.

Further identification of components of the Tc model developed for man (Hays) with anatomical and physiological mechanisms has been obtained. Parameter values related to intestinal transports were found to change probably due to ambulatory state changes of patients.

Significance for Cancer Research: Modeling provides a means for detailed analysis of metabolic systems in normal and abnormal states. For example, lithium is now being used to suppress thyroid hormone release during radiotherapy of thyroid cancer and hyperthyroid patients. The fetal model will give further insight into placental transport of pharmacologic agents as well as normal metabolites. Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: 1) Continued development of fetal system, 2) continued development of TRH and TSH models, 3) integration of TRH and TSH models into the thyroid model.

Keyword Descriptors: Modeling, thyroid, lithium, thyroxine, triiodothyronine, fetus, amniotic fluid, placenta, thyrotropin, thyrotropin releasing hormone.

Publication:

Belshaw, B.E., Barandes, M., Becker, D.V., and Berman, M.: A Model of Iodine Kinetics in the Dog. Endocrinology. 95: 1078-1093, 1974.

Singh, B., Sharma, S.M., Patel, M.C., Raghavendran, K.V., and Berman, M.: Kinetics of Large Therapy Doses of ^{131}I in Patients with Thyroid Cancer. J. Nuclear Medicine. 15: 674-678, 1974.

Project No. Z01 CBD 08317-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Theory of Conformational Transitions in Polypeptides

Previous Serial Number: 5.2 (DCRT)

Principal Investigator: Robert Jernigan

Other Investigators: Shousun Szu

Cooperating Units: James Ferretti (DCRT/PSL)

Man Years:

Total:	1.4
Professional:	1.4
Other:	0

Project Description:

Objectives: To elicit molecular details of conformational kinetics in polypeptides. An eventual aim is to understand the mechanisms and pathways of protein folding and denaturation-renaturation kinetics.

Methods Employed: A mathematical model of the rates for passing between the various configurational states has been established. Molecular states are determined by specification of whether each peptide unit has a helical or random coil conformation.

Major Findings:

Kinetics of configurational transitions can be followed for polypeptides of any specified sequence of amino acids. We have accounted for frictional resistance to internal transitions. This effect slows the transition rates but does not affect the shapes of the transition curves.

Significance for Biomedical and Cancer Research: Development of a basic understanding of the syntheses and foldings of proteins into native states is essential to understanding cellular activities on a molecular basis. Such comprehension is necessary before detailed effects of external chemical agents can be determined. This is pertinent to Objective 1, Approach 2 and Objective 4, Approach 4 of the National Cancer Plan.

Project No. Z01 CBD 08317-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Proposed Course of Study: The model has served to point out some needed experiments. Careful interpretation of experiments will yield individual peptide transition rates for a range of amino acids and permit treatment of specific proteins. Additional states will be included in a modified model in order to incorporate additional configurational forms that are observed in proteins. Many physical properties of proteins can then be calculated.

Key Word Descriptors: Configuration, conformation, polypeptides, kinetics, helix, random coil, solvent, friction, protein, renaturation.

Publications:

Jernigan, R.L., and Ferretti, J.A.: Mean configurational relaxation rates in finite length polypeptides. J. Chem. Phys. 62: 2519-2529, 1975.

Jernigan, R.L., and Ferretti, J.A.: Conformational relaxation times in the polypeptide helix-random coil transition region. Submitted to J. Mol. Biol.

Project No. Z01 CBD 08318-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Molecular Mechanics

Previous Serial Number: 5.3 (DCRT)

Principal Investigator: Robert Jernigan

Other Investigator: Shousun Szu

Cooperating Units: George Weiss (DCRT/PSL)

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: The purpose is to predict dilute solution behavior with detailed molecular models for internal macromolecular conformational transitions.

Methods Employed: Experimental information on small molecules is combined with a one-dimensional configurational model to permit calculation of properties of large linear molecules. Each chemical bond is permitted to assume several fixed geometry rotational isomeric states. Nearest neighbor dependences for transition rates between these states are included. The theory is valid for linear molecules of any length.

Major Findings:

Previous theories for calculating time correlation functions did not properly account for molecular symmetries. Rates for relaxation of each atom can be calculated and directly compared with NMR relaxation measurements for simple carbon atom chains.

Significance to Biomedical and Cancer Research: These calculations allow a prediction of a variety of physical properties for linear molecules of any given monomer sequence. Such calculations in advance of laboratory experiments can provide guidance to the experimental scientist. This is consistent with Objective 4, Approach 4 of the National Cancer Plan.

Project No. Z01 CBD 08318-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Proposed Course of Study: Calculations of several different time correlation functions are planned. We would like to determine whether or not different relaxation measurement methods yield similar relaxation times.

Keyword Descriptors: Macromolecule, configuration, conformation, transitions, NMR, kinetics, time correlation functions, and rotational isomers.

Project No. Z01 CBD 08319-01 LTB
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Population Genetic: X-Linked Transmission

Previous Serial Number: None

Principal Investigator: Paul Van Eerdewegh

Other Investigators: Michele Van Eerdewegh (NIMH)
Elliot Gershon (NIMH)

Cooperating Unit: Section on Psychogenetics
Adult Psychiatry Branch, NIMH

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: Dealing with phenotypic traits which are the expression of genotypes at a single locus on the X-chromosome, we develop a model of genetic transmission which takes into account a random environmental factor: this random environmental effect being responsible for the incomplete penetrance of the genotypes. In other words a given genotype has no longer a unique phenotypic expression and a given phenotype could be the expression of any genotype at this single locus.

We derive the covariance between relatives under X-linked transmission.

Major Findings: The covariance between relatives allows us to find, for any model of transmission, what is the maximum number of parameters that can usefully be fitted when the data are prevalences among relatives of probands.

This model of transmission has been applied to human data; namely on affective illness and a single major X-linked gene can account for the bipolar affective disorder.

Significance to Cancer Research: This kind of approach can be used for testing the hypothesis of a single gene (with complete or incomplete penetrance) factor in the transmission of qualitative or quantitative traits and also to predict the prevalence of a trait among the relatives of a proband. This closely fits into Objective 2, Approach 5 of the National Cancer Plan.

Keyword Descriptors: Population genetic, X-linked single locus transmission, incomplete penetrance, covariance between relatives, affective illness.

IMMUNOLOGY BRANCH

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SUMMARY

Annual Report of the Immunology Branch, National Cancer Institute
July 1, 1974 through June 30, 1975

INTRODUCTION

The Immunology Branch carries out basic immunobiology laboratory investigations with particular emphasis given to studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response and transplantation. The fundamental information obtained is then applied to investigations of the interactions between the immune system and neoplastic cells, as well as to the possible diagnosis and therapy of malignant disease in animal models and in man.

I. STRUCTURE AND FUNCTION OF IMMUNE CELL AND NEOPLASTIC CELL MEMBRANES AND CELL PRODUCTS

A. Membrane Receptors. The function of cells, including cells of the immune system, is partially initiated and modulated by interactions between cell surface receptors and a variety of ligands. One such receptor on the B cell subpopulation of human lymphocytes is a receptor for antigen-antibody complexes or for aggregated IgG (5035). This receptor has been partially characterized and been shown to be distinct from surface Ig and the C3 receptor. It is specific for the Fc portion of complexed Ig and the site on the Fc portion of the immunoglobulin molecule involves a stabilizing disulfide bond. The Fc receptor on B cells is necessary for antibody dependent cell mediated cytolysis (see II, B). The Fc receptors of murine B lymphocytes are either identical to or closely associated with Ia cell surface antigens (5021, IV, A). T lymphocytes also appear to have Fc receptors but they are present in lower numbers or have a lower affinity for immune complexes than do those of B cells.

The Fc receptor has been used to develop a preparative technique for separating human lymphocytes into T and B cell populations (5018). Plastic surfaces are coated with antigen-antibody complexes and these coated surfaces selectively retain human peripheral B cells which contain Fc receptors. Binding of lymphocytes to antibody coated plastic surfaces does not trigger an increase in DNA or protein synthesis. It does, however, appear to inhibit the mitogenic effect of lypopolysaccharide and con A, but not that of PHA. Thus, when B cells bind to immune complexes on a plastic surface, the membrane receptors for lypopolysaccharide and con A are either sterically blocked or the proliferative signals are suppressed.

Attempts are being made to isolate and characterize the Fc receptor (5048).

B. Mechanisms of Cell Killing. Studies are being performed in an attempt to understand the mechanisms by which lymphocytes kill target cells (5018). Lipid bilayer membranes have been used as a model for the target cell membrane. When these model membranes contain DNP groups, and antibodies for DNP is allowed to interact with the membrane, subsequent addition of non-immune human lymphocytes causes a conductance increase across the antibody coated lipid bilayers of several orders of magnitude. These results suggest that an increase in ionic permeability may be the basic mechanism responsible for target cell death, at least in the antibody dependent cell mediated killing system. This model provides an exceedingly useful approach to determining the mechanisms by which membrane damage may be caused. Previous studies in the antibody dependent cell mediated killing system have shown that attacking cells undergo a marked shape change when they interact with target cells. Lymphocytes that interact with antibody coated plastic surfaces undergo identical rapid and dramatic morphologic changes. These changes, and the capacity of these cells to kill in the antibody mediated system, can be blocked by a variety of drugs. These studies suggest that the morphologic changes are a necessary part of the lymphocyte killing process and permit this process to be divided into a phase of morphologic change which is then followed by the actual killing phase.

C. Normal Cell Surface Antigens. Human HL-A antigens have been further characterized (5006). Detergent solubilized HL-A antigens have been found to have a molecular weight of 44,000, as contrasted to the HL-A material that can be isolated after treatment of cell membranes with papain. This latter material has a molecular weight of 34,000. Both the detergent solubilized and papain solubilized products are associated with β_2 microglobulin.

New antigens are being detected on human B lymphoid cells, and by analogy, these antigens are likely to be comparable to the Ia antigens of mouse B cells. In one system (5006) sera obtained from multiparous members of the inbred religious sect, the Amish, have been shown to detect antigens expressed on B cells. These antigens have been shown to be serologically different from HL-A alloantigens. They differ as well chemically and have an apparent molecular weight of approximately 30,000.

In a different approach to this problem (5034), pretreatment of human lymphocytes with appropriately absorbed anti-HL-A antisera inhibits the binding of Ig complexes to the Fc receptors of B lymphocytes. In the mouse system, it has been shown that pretreatment of B cells with serum containing anti-Ia, but not anti-H-2, similarly inhibits binding of Ig complexes to Fc receptors (5035). It is presumed that both the sera obtained from multiparous Amish women and the specifically chosen HL-A antisera contain antibodies with specificity for human cell surface Ia antigens.

Human lymphoid cells have been further characterized in terms of cell surface markers (5034). Peripheral blood lymphocytes from normal humans were found to consist of four subpopulations. The use of cell surface markers has permitted the separation of patients with variable immunodeficiency and panhypogammaglobulinemia into distinct groups that appear to differ as regards the nature of their fundamental defect. Patients with chronic lymphocytic leukemia could be separated into T and B cell types.

D. Tumor Cell Antigens. Lymphoid cells from patients with acute lymphocytic leukemia appear to contain a cell surface antigen not found on lymphoid cells. Antiserums prepared in rabbits detect this antigen (5006). Serums from patients receiving allogeneic leukemia cells, however, were found to possess antibody predominantly to HL-A antigens and no antibody to leukemia associated antigens was found.

E. Modified Cell Surfaces. Treatment of cell surfaces with the enzyme neuraminidase alters them in such a way that they react with a "natural" antibody present in normal human serum and in some mouse serums (5017). The serum concentration of this antibody was studied in patients with newly diagnosed malignancies and in patients with various forms of immunodeficiency. The distribution of antibody levels in these patients did not differ from that of age matched control normals. It appears that low levels of this antibody is not involved in a predisposition to the development of cancer and high levels do not prevent the development of cancer. Studies were also performed to determine the fate of labelled neuraminidase treated autologous lymphocytes in mice known to contain high levels of antibody reactive with neuraminidase treated cells. No important differences in the *in vivo* fate was detected when neuraminidase treated cells were infused into either high or low antibody strains. The biologic role of this "natural" antibody therefore remains to be elucidated.

II. CELLULAR IMMUNITY

A. Separation and Characterization of Lymphoid Cell Populations. Separation of subpopulations of normal mouse spleen cells using nylon column passage, has revealed synergy between what are presumed to be T and B cells in the generation of cell mediated cytotoxicity (5039). An analysis of the cells required for stimulating the induction of *in vitro* sensitization for cytotoxicity indicates that either T cells or B cells may serve as adequate stimulators.

Murine lymphoid cells have been separated on sepharose columns coated with histamine (5028). Cells of differing functional characteristics can be separated by this procedure. Opposite effects on *in vivo* antibody production to sheep erythrocytes and on *in vitro* cell mediated immunity to alloantigens were found and it will be necessary to develop a system in which cell mediated and humoral immunity to the same antigenic determinants can be induced in order to compare the histamine column effect on *in vivo* and *in vitro* aspects of these immune parameters.

Functionally different subpopulations of mouse T lymphocytes have been identified (5003). Primary *in vitro* sensitization of spleen cells against NIP-modified syngeneic cells generates cytotoxic cells specific for cells modified with the hapten NIP. *In vivo* sensitization with NIP-modified syngeneic cells generates memory cells but no cytotoxic cells. These experiments suggest that cytotoxic T cells are a different population from the T cells that express memory for cytotoxic response.

B. Characterization of Lymphoid Populations of AKR Mice. An attempt is being made to define the defect in the lymphoid system of AKR mice which predisposes to the high spontaneous incidence of thymic lymphoma and lymphocytic leukemia (5041). It has been shown that spleens and lymph nodes of leukemic animals contain an increased proportion of theta positive cells. In addition, there is a disproportionately greater loss of con A responsiveness than PHA responsiveness in preleukemic and leukemic AKR mice. This leads to a progressively greater PHA to con A stimulation ratio as animals age and become leukemic. These results suggest that there is a con A responsive subpopulation of cells and that this population is more readily inactivated by murine leukemia virus (5041). Additional studies are being performed on the characterization of leukemia cell lines derived from AKR mice that has been thymectomized neonatally (5043). A large percentage of the cells derived from these animals have both T and B cell surface markers and it may permit the identification and study of a transitional subpopulation involved in the differentiation of lymphoid cells.

C. Antigen Induced Changes in Lymphocyte Trapping. Lymphocytes exposed to antigen *in vivo* localize preferentially in spleens or lymph nodes (5042). This phenomenon is termed trapping and it has been shown to be a T cell dependent phenomenon. The lymphocyte populations involved in the trapping phenomenon have been studied and the results indicate that T cell subpopulations, particularly in the spleen, are capable of bidirectionally augmenting or suppressing the magnitude of lymphocyte trapping following antigenic challenge.

D. Antibody Dependent Lymphocyte Mediated Cytotoxicity. The class of antibody required to sensitize target cells has been studied further (5006). Sera fractionated by gel filtration and ion exchange chromatography produce results indicating that cytotoxic antibody can be separated from lymphocyte dependent antibody. It is not known whether these represent functional differences amongst subclasses of IgG or some other factor. The effector cell in this system has been studied in a number of ways. Human T cells are separated from B cells by forming rosettes and separating cell populations in a gradient. These studies suggest that cells with B characteristics were primarily responsible for target cell destruction. A different technique for separation, that of adherence to antigen-antibody coated plastic surfaces, shows that nonadherent cells (primarily T cells) are severely depleted of killing activity in the antibody dependent killing reaction (5018).

The role of the antibody dependent cell mediated cytotoxicity system in tumor bearing animals has been explored (5003). Lymphoid tumors in several different mouse strains were capable of inducing a peritoneal exudate which contained effector cells for antibody dependent cellular cytotoxicity. These experiments paved the way for more detailed analysis of the *in vivo* significance of this form of cell killing.

III. GENETICS OF THE IMMUNE RESPONSE

The demonstration of genetic control of immune responses has opened an important pathway in gaining an understanding of the biology of the immune system. A variety of studies are being carried out in an attempt to understand the nature of the genetic control as well as to determine the biologic significance of this type of control.

A. Genetic Control of Immune Responses Linked to H-2. In studying genetic control of immune responses, it is desirable to utilize antigens of known structure. Staphylococcal nuclease meets this requirement. H-2 linked genetic control of antibody synthesis has been demonstrated and localized to the Ir I-B region (5025). Cell mediated immunity to this antigen measured in vitro, was also shown to be under genetic control (5026).

The genetic control of cell mediated immunity to synthetic polypeptides and to staphylococcal nuclease was also determined with an in vivo assay. It was clearly demonstrated that cell mediated immunity is under genetic control and that the genes concerned are H-2 linked Ir genes. The in vivo delayed hypersensitivity was transferable by T cells but not by B cells from the immunized animals (5046).

B. Genetic Control of Immune Responses Not Linked to H-2. Non-H-2 linked antibody responses have been demonstrated for lysozyme and synthetic polypeptides (5027). The ability of irradiated mice to reject hamster bone marrow transplants is also under non-H-2 linked genetic control. Studies have been shown that at least one but not more than two non-H-2 linked genes control this rejection capacity.

C. Induction of In Vitro Cell Mediated Immunity Against Hemopoietic Histocompatibility Antigens. Hemopoietic histocompatibility antigens are phenotypically expressed only on blood forming and leukemic cells and they are capable of stimulating host versus graft reactions which are radioresistant in vivo. It is now been possible to develop an in vitro system in which F1 responding lymphocytes generate cytotoxic effector cells against spleen cells of H-2^b parents but not against H-2^a parents (5049). This is the first demonstration of such in vitro activity and should make it possible to experimentally explore this complex system of antigens which are regulated by multiple H-2 linked genes.

D. Genetic Control of Responses to Altered Cell Surface Antigens. Cell mediated immunity can be induced in vitro against hapten modified target cells. This system demonstrates two aspects of genetic control (5038, 5045, 5003). It has been shown that the antigen developed on the cell surface consists of a hapten modified protein which is itself an H-2 antigen under the genetic control of either the D or K end of the H-2 complex. The immune response is not directed against the hapten itself but rather against the hapten-modified cell surface proteins. The capacity to develop an immune response to the hapten-modified cell surface protein is also under genetic control which is H-2 linked and which maps to the left of the Ia genetic region.

E. Relationship Between Ir Genes and Immunoglobulin Variable Region Genes. Studies are being carried out to determine whether Ir genes control the immunoglobulin variable regions that are produced in "genetically controlled" antibody responses (5036). Specifically purified mouse antistaphylococcal nuclease antibodies have been prepared and used to immunize rats. Rats have made antibodies against the mouse antibodies and by several criteria, these antisera have been shown to contain antibodies specifically directed against idiotypic determinants. These rat antibodies have the capacity to react with the combining site of the mouse antistaphylococcal nuclease antibodies as evidenced by their ability to inhibit the capacity of the mouse antibody to inactivate staphylococcal nuclease in an enzymatic assay.

IV. TRANSPLANTATION IMMUNOBIOLOGY

A. Antigens of the Major Histocompatibility Locus. Further work has been done on characterizing Ia antigens and their role in immunologic responsiveness. Ia antigens have been shown to be preferentially expressed on B cells and appear to be associated with or responsible for the stimulation of mixed lymphocyte culture reactions (5021). The use of more sensitive techniques, including the fluorescent activated cell sorter, have demonstrated that Ia antigens are also present on at least some T cells (5035).

Anti-Ia antibodies have been found to specifically block the Fc receptor of B lymphocytes (5021, 5035). This has led to the hypothesis that Ia antigens and the Fc receptor are either identical cell surface components or that they are physically located so closely together that immunologic reactions at the site of one of these components sterically blocks the functional reactivity of the other.

The structure of Ia antigens has been studied using specific immune precipitation followed by analysis on polyacrylamide gels (5047). Specific antisera for Ia antigens has been used to determine whether specificities are of the same or different molecules. Specificities 8 and 11, which map in the I-A subregion, were shown to be located on the same molecule, as were specificities 8 and 9. By contrast specificities 5 and 7, which map in the I-C region, are located on separate molecules.

B. Transplantation Antigens of Swine. The breeding program which was begun last year has continued successfully (5023). Selected third and fourth generation animals have been reciprocally skin grafted and this has led to the development of large quantities of high titered antiserum with histocompatibility specificity. Fourth generation offspring have yielded three breeding pairs of animals homozygous for serologically determined transplantation antigens, thus establishing the capacity to breed three different herds of swine each homozygous for a different SL-A antigen. MLC assays have shown close correlation with serologically determined histocompatibility antigens. This indicates that the breeding is selective for homozygosity at a major histocompatibility complex homologous to that of other mammalian species.

C. Pre-clinical Model Bone Marrow Transplantation. Rhesus monkeys are being used to study allogeneic bone marrow transplantation and graft versus host disease (5011). Newer modes of immune suppression of allografts have been further studied. Additional rhesus histocompatibility antigens have been discovered. One animal has been identified which may be homozygous at the MLC locus. If this can be verified it would provide an important lead in defining the products of the MLC locus. Ia antigens are being looked for and some antisera have been developed which block the capacity of B cells to bind aggregated immunoglobulin.

D. HL-A and Disease Associations. Patients with various diseases are HL-A typed and associations are sought between specific antigens and disease (5017). An interesting observation has been made in lung cancer. Patients with the antigens HL-A5 and/or W19 had a sixty-percent change of surviving disease free for one year post-therapy while patients who lacked these antigens had only a fifteen percent chance for surviving one year disease free. Attempts are being made to extend this study prospectively in order to confirm the observation.

V. STRUCTURE AND IMMUNOCHEMISTRY OF IMMUNOGLOBULINS

A. Three Dimensional Structure of Immunoglobulins. Further analysis has been carried out on the interaction between the phosphorylcholine antigen and the combining site of a mouse myeloma protein that has been crystalized (5040). There are strong interactions between the phosphate group of the hapten and the side chain groups of tyrosine 33 and arginine 52, both of the heavy chain. Acedic side groups from both light and heavy chains are in the immediate vicinity of the positively charged choline group. Comparative studies have been performed on the variable domain of a number of crystallized myeloma proteins. Based on these observations hypotheses have been established concerning the way in which antigenic recognition takes place on molecular surfaces that involve relatively invariant framework regions and more highly variable loops.

B. Idiotypic Studies of Human Myeloma IgA. The myeloma protein of a young patient with myeloma has been isolated and idiotype antisera prepared (5022). These antisera have been used to monitor quantitative levels of myeloma protein in the patient as a means of monitoring his therapy. The patient continues in clinical remission but low levels of idiotype are still detectable in his serum. This idiotype could not be detected in the serum of forty-seven other patients with IgA myeloma nor could it be detected in the patients to parents or for siblings.

VI. CLINICAL IMMUNOTHERAPY

A study of the immunotherapy of melanoma has been in progress for several years (5033). A study of patients with stage III disease has been terminated after thirteen patients were placed into the chemotherapy arm of the study and sixteen into the immunotherapy arm. There were no striking significant differences between either of these therapies alone. A study of patients with stage II melanoma has been carried out in an exploratory manner. This study is being revised and a new protocol will be started in the near future.

Serial No. NCI Z01-CBD 5003(c)-09 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular Immunity I

Previous Serial Number: Same

Principal Investigator: John R. Wunderlich, M.D.

Other Investigators: Arnold E. Eggers, M.D., Hillel S. Koren, Ph.D., David Steinmuller, Ph.D., Susan O'Hopp Sharrow

Cooperating Units: Janet W. Hartley, Ph.D., Laboratory of Viral Diseases NIAID, and John K. Inman, Ph.D., Immunochemistry Section, Laboratory of Immunology, NIAID, Elizabeth Simpson, DVM, Surgical Sciences, Clinical Research Center, England

Man Years:

Total:	5
Professional:	3
Other:	2

Project Description:

Objectives: This project has been directed at understanding and manipulating mechanisms of cell-mediated immune cytotoxicity. Long-range goals are: (1) to identify and characterize factors which influence induction, maturation, and expression of a cellular immune response; (2) to define changes in cellular immunity which occur in tumor bearing hosts; and (3) to find means of manipulating the cellular immune response for therapeutic purposes. Immediate objectives of the past year have been to concentrate on goals 2 and 3.

Methods Employed: Direct, cell-mediated cytotoxicity has been measured in vitro by release of ^{51}Cr from labelled target cells incubated with immune lymphoid cells for 2-4 hours. Immune cells are collected from spleen marrow lymph node, thymus and peripheral blood of sensitized hosts. Several techniques have been used for fractionating cells. Spleen cells have been fractionated on nylon columns; effluent cells are highly enriched for T lymphocytes. In addition, immune cells have been produced by in vitro sensitization of spleen cells from normal donors using previously established Mishell and Dutton tissue culture techniques. Target cells are provided by freshly explanted tumors, tumor tissue culture lines, and normal lymphoid lines.

Target cells have also been chemically modified not only for use as target cells in ^{51}Cr release assays, but also for use as in vitro immunogens. Chemical modification has been primarily with NIP and arsonyl conjugates. These reactants are particularly advantageous in that they combine covalently with cell surfaces under physiologically mild conditions.

Major Findings: 1) Functionally different subpopulations of mouse T lymphocytes have been identified which recognize hapten-modified, syngeneic cells. Thus, primary in vitro sensitization of spleen cells against NIP-modified syngeneic cells generates cytotoxic cells specific for the hapten NIP. On the other hand, in vivo sensitization of mice with NIP-modified syngeneic cells generates hapten-specific "memory" T cells but no cytotoxic cells. Memory cells are defined as those cells capable of giving a secondary type of cytotoxic T cell response following exposure to antigen in vitro.

2) Effector cells dependent on humoral antibody have been identified in mouse peritoneal exudates induced by syngeneic tumors. Thus lymphoid tumors of three different mouse strains, AKR, BALB/c, and C57BL/6, were each capable of growing in ascites form and inducing a peritoneal exudate which contained effector cells for antibody-dependent cellular cytotoxicity (ADCC). Several findings stand against the possibility that these tumor cells per se act as effectors in ADCC. Only one of six tumors functioned as an effector cell after separation from host cells by in vitro passage. Furthermore, one of the tumors lacks Fc receptors and thus could not act as an effector cell in ADCC. These findings support the concept that host lymphoid cells capable of serving as effector cells in ADCC comprise part of the exudate associated with ascitic murine tumors.

3) The Fluorescent Activated Cell Sorter, which is maintained by our laboratory, has provided nearly all of the data for two independent studies. Fathman, et al., have identified Ia antigens on mouse thymocytes using detection sensitivities not available with visual fluorescent microscopy. Scher, et al., have identified a genetically controlled B lymphocyte deficit in CBA/HN mice by studying cell frequency distribution histograms based on cell surface immunoglobulin.

Significance for Cancer Research and the Program of the Institute: (1) Various subpopulations of sensitized cells capable of recognizing haptens are being used in our laboratory in an attempt to increase the immunogenicity of syngeneic tumors. The rationale behind this approach is based upon the concept of "heterogenization" in which new antigens are introduced onto tumor cell surfaces to help present the original tumor antigens to the host immune system. (2) Identification of effector cells for antibody-dependent cellular cytotoxicity in ascitic tumor fluid provides an additional means for studying immunocompetent host cells present at a tumor site. (3) The relevance of Ia antigens on T cells has been presented in Dr. Sachs' report. Understanding the nature of B cell lymphocytes is important for determining the role of humoral antibody responses to tumors. We intend to use this deficient mouse strain in several tumor model systems. This work supports Objective 6, Approach 4.

Proposed Course of Project: Primary effort will be directed at increasing the *in vitro* cytotoxic murine T cell response against syngeneic tumors. A variety of approaches will be used including: cell fractionation techniques previously demonstrated by us to be effective in removing suppressor cells and enriching responder cells from tumor-bearing hosts; hapten modification of tumor cells and use of presensitized antihapten "helper" cells; and presensitization of responder cells against unrelated antigens previously demonstrated by us to improve the cytotoxic immune cell response of AKR lymphoid cells against syngeneic leukemia cells.

Honors and Awards: None

Publications:

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lymphocytes to antibody treated target cells. Cellular Immunol., in press.

Yust, I., Wunderlich, J. R., Mann, D. L., and Terry, W. D.: Identification of lymphocyte dependent antibody in sera from multiply transfused patients. Transplantation 18:99-107, 1974.

Yust, I., Wunderlich, J., Mann, D. L., Leventhal, B., Yankee, R., and Graw, R.: Human lymphocyte dependent antibody mediated cytotoxicity against non-HL-A antigens. Nature 249:263-265, 1974.

Key Words: Tumor cell heterogenization; T cell specificity; fluorescent activated cell sorting; antibody-dependent effector cells.

Serial No. NCI-Z01-CBD 5006(c)-09 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: I) Transplantation and Associated Cell Membrane Antigens
II) Acute Leukemia Associated Antigens
III) Characterization of Lymphocyte Dependent Antibody

Previous Serial Number: Same

Principal Investigator: Dean L. Mann, M.D.

Other Investigators: June Miller, Ph.D., John Wunderlich M.D.

Cooperating Units: Brigid G. Leventhal, M.D., Pediatric Oncology Branch, DCT, NCI

Man Years:

Total: 3
Professional: 3
Other: 0

Project Description:

Objectives: I - Transplantation and Associated Cell Membrane Antigens: The objectives of the studies are fourfold: a) to purify human cell surface antigens; b) to chemically, genetically and immunologically characterize these antigens; c) to use soluble human cell membrane antigens to raise allotypic antisera in heterologous species; d) to define and characterize cell surface components that have functional significance and relate them to HL-A alloantigens. This work supports Objective No. 3, Approach No. 5.
II - Acute Leukemia Associated Antigens: a) identification of an antigen associated with acute leukemia; b) isolation and characterization of this antigen; c) use antigen to produce heterologous antiserums; d) identify factor(s) in serum of leukemic patients that inhibit and/or promote cell-mediated cytolysis; e) assess the immunologic response of patients to leukemia associated antigens after immunization with the Raji cell line. This work supports Objective 6, Approach 3. III - Characterization of Lymphocyte Dependent Antibody: A serum component (antibody) mediating cellular cytotoxicity (LDA) will be identified and characterized. Antisera possessing this activity will be separated from antibody causing complement-mediated cytotoxicity using physical and immunological techniques. The nature of the antigen reacting with this antibody will be explored. The specific population of lymphocytes participating in this reaction will be identified. This

work supports Objective No. 6, Approach 4.

Methods Employed: ^{51}Cr release and dye exclusion cytotoxicity tests (See Project Report NCI-5008) were used to detect cell surface antigens. Human cells were obtained from normal blood bank donors and from long term tissue culture. Antisera detecting genetically determined human leukocyte alloantigens were obtained as described in Project NCI-5008. In addition, human and rabbit sera reacting with these cells but not containing HL-A antibodies were used. Alloantigens in subcellular form were detected by a cytotoxic inhibition assay using the ^{51}Cr release from target cells and employing the antiserums described above.

Human leukocyte alloantigens were prepared by graded hypotonic lysis of cell suspensions. Membrane fragments were treated with papain or detergents to yield a water soluble antigen. Further purification was done by CM-Sephadex chromatography, gel filtration chromatography, electrofocusing and acrylamide gel electrophoresis. The physical and chemical properties of the antigens were investigated.

To aid in studies of identification and isolation of HL-A alloantigens, and other cell surface antigens, heterologous antiserums were raised by injecting purified or partially purified cell membrane component into rabbits. These antiserums were evaluated for cytotoxicity to human lymphoid cells as well as for their ability to precipitate solubilized cell membrane components. The precipitation techniques were utilized to isolate HL-A bearing alloantigens as well as non-HL-A cell surface antigens. Quantities of lymphoid cells needed to pursue this investigation will be provided by NCI contract NO1-CB-12078.

The acute leukemia associated antigen(s) was detected in dye exclusion and ^{51}Cr chromium release cytotoxicity assays. Antiserum used in these studies was obtained by immunizing rabbits with water soluble cell membrane preparations from the tissue culture cell line Raji as well as serum from patients immunized with Raji cells. Peripheral blood lymphocytes from patients with acute leukemia were labeled with ^{51}Cr, incubated with autologous serums or serum from other leukemic patients and/or with heterologous antiserum that detect the acute leukemia antigen. Normal peripheral blood lymphocytes were added and the lysis or inhibition of lysis mediated by the various serums determined. Patients injected with the Raji cell lines were assessed for the development of LDA and complement dependent cytotoxic antibody. Serums from patients who had received immunization with allogeneic leukemia cells were screened for antibody to HL-A and/or leukemia associated antigens.

Serums were obtained from patients who had received multiple transfusions or had a diagnosis of autoimmune disease or malignancies. These serums were tested in the antibody mediated cellular cytotoxicity reaction using various target cells and normal peripheral blood lymphocytes. The positive sera were fractionated by gel filtration, ion exchange chromatography, and electrofocusing. These fractions were tested for LDA to determine the immunoglobulin component responsible for this antibody activity. The subpopulation of lymphocytes responsible for target cell destruction in the LDA reaction was investigated. Peripheral blood lymphocytes were separated on the basis of their T and B characteristics and used to determine which population caused target cell destruction.

Major Findings: I - Transplantation Antigens: Several methods were used to prepare soluble cell membrane antigens (HL-A) from tissue culture cell sources. Membrane materials solubilized with nonionic detergents were fractionated to ascertain the molecular characteristics of the HL-A alloantigens. The products were compared with those materials isolated by papain digestion. Immune precipitation tests were developed and used to isolate the HL-A containing membrane substances. The antigen-antibody complexes were dissociated and the antigen characterized on disc gel electrophoresis. Both the detergent solubilized and the papain solubilized products carried the β-2 microglobulin. Size differences in HL-A antigens solubilized by detergents and papain could be accounted for by the action of the enzyme on the native product. Detergent solubilized HL-A antigens were found to have a molecular weight of 44,000. Papain treatment of this material resulted in a product with a molecular weight of 34,000, the same as would result by treatment of the cell membrane with papain. Milligram quantities of purified HL-A antigens are being prepared for amino acid sequence analysis.

Serologic reagents have been identified that detect allotypic antigens on human "B"-lymphoid cells. These reagents have been procured from multiparous from an inbred religious sect, the Amish. Sera, showing no reactivity to HL-A alloantigens, detect antigens expressed on lymphoid cells with "B" characteristics. Using precipitation techniques, these cell surface components appear to have a molecular weight of ∿30,000 in contrast to HL-A antigens with a molecular weight of ∿44,000.

II - Acute Leukemia Associated Antigens: Antiserums obtained from rabbits immunized with the soluble cell membrane component from the cell line Raji were found to detect an antigen which is associated only with acute leukemia cells. Serums obtained from patients with acute leukemia and immunized with the Raji cell line were found to have the same pattern of reactivity as the rabbit strain. These serums were used to separate the leukemia antigens from the HL-A antigens of the Raji cell line.

Serums from patients receiving allogenic leukemia cells were found to possess antibody predominantly to HL-A antigens. No antibody to leukemia associated antigens was found.

III - Characterization of Lymphocyte Dependent Antibody: In studies attempting to isolate and characterize LDA, it has been demonstrated that patients receiving multiple transfusions have highest titers of this serum component. These sera were found to react to non-HL-A antigens. Sera were fractionated by gel filtration and ion exchange chromatography as well as electrofocusing. Studies have indicated that cytotoxic antibody can be separated from LDA using combinations of a number of different sera and target cells. The LDA appears to be reacting in some instances with a non-HL-A antigen. Lymphoid "T" cells and "B" were separated using rosetting techniques and tested for their ability to cause destruction of target cells coated with LDA. Cells with "B" characteristic were found to be primarily responsible for this destruction.

Significance to Biomedical Research and the Program of the Institute:
I-Transplantation Antigens: The preparation and analysis of soluble human transplantation antigens has both fundamental and clinical importance. Information as to the character of these antigens and the nature of the integration of these materials into the cell membrane structures will add to our knowledge of genetic control as well as fundamental knowledge in basic cell membrane structure. Such studies using known markers on cell surfaces become the prototype for investigating tumor antigens. There is evidence suggesting the association of HL-A alloantigens to some neoplastic disease. If the non-HL-A alloantigens are genetically linked to HL-A, reagents identifying these antigens may more definitively define this association. The relationship of these antigens (cell surface structures) to the ability to respond immunologically to foreign materials has fundamental biologic importance.

II - Acute Leukemia Associated Antigens: The demonstration that acute leukemia cells possess what appear to be specific antigenic determinants gives a rational basis for immunotherapeutic trials. Studies thus far indicate that patients immunized with Raji develop antibody to leukemia cells. However, this reactivity is lost when the patient's disease relapses. The protocol has been extended to combination chemotherapy and immunization in attempts to maintain an immunologic response to these antigens.

III - Characterization of Lymphocyte Dependent Antibody: The isolation and characterization of the lymphocyte dependent antibody is a relatively new area for exploration. The LDA may represent a new mechanism for destruction of tissues by the host's own lymphocytes. Investigation of the nature of this antibody and its specificity of reaction should contribute to our knowledge of immune reactions in patients with autoimmune disease as well as those individuals who possess malignancies.

Proposed Course of Project: Further chemical and biologic characterization of soluble HL-A alloantigens will be undertaken. Quantities of purified HL-A alloantigens have been prepared and amino acid sequence studies are underway. Alloantigens specific for "B" cells will be investigated on a molecular basis as well as the assessment of genes controlling the expression of these antigens. Attempts will be made to determine the biologic significance of the cell surface structure both in malignancy and in the normal immune response.

Studies will continue in the isolation and characterization of the antigen associated with acute leukemia cells. Patients undergoing chemoimmunotherapy or chemotherapy both will be studied for their immune response. Studies to be performed include assessment of antibody production, direct cellular cytotoxicity, and lymphocyte migration inhibition. The relationship of immune response of these patients to remission duration will be assessed.

Further studies will be undertaken in an attempt to identify the specific serum component responsible for the LDA activity. Studies are underway to identify the specific cell surface antigen responsible for the production of this antibody. Heterologous antisera that blocks the attacking cells have been developed. The nature of the receptor on the cell surface of the attacking cell will be investigated using this antisera.

Honors and Awards: None

Publications:

Mann, D. L., Halterman, R., and Leventhal, B.: Acute leukemia associated antigens. Cancer 34:1446-1451, 1974.

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Key Words: Transplantation antigens; B cell antigens; Isolation; Biochemical characterization; Leukemia associated antigens; Immunotherapy; Lymphocyte dependent antibody.

Serial No. NCI Z01-CBD 5011(c)-05 I
1. Immunology Branch
2. Transplantation Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Preclinical Immunological Studies in Bone Marrow Transplantation

Previous Serial Number: Same

Principal Investigator: John R. Neefe, Jr., M.D.

Other Investigators: G. Nicholas Rogentine, Jr., M.D.

Cooperating Units: Robert G. Graw, Jr., M.D., Pediatric Oncology Branch, DCT, NCI

Contractor: Litton Bionetics Research Laboratory
Contract N01-CB-02103

Man Years:

Total:	1 1/4
Professional:	1 1/4
Other:	0

Project Description:

Objectives: 1) To investigate newer forms of immune suppression of allograft rejection and the graft-versus-host reaction (GVHR) in a rhesus monkey bone marrow transplant model prior to clinical use; 2) to identify monkey transplantation antigens; 3) to establish a colony of pedigreed rhesus families to study the genetics of transplantation antigens and eventually to provide a source of related donor-recipient pairs for bone marrow transplantation.

Almost all of this work is being conducted by a close collaboration with Litton Bionetics under contract N01-CB-02103. Dr. Darrow is the Principal Investigator and Dr. Rogentine the Project Officer. Dr. Neefe and Dr. Graw are Co-Project Officers.

Methods Employed: Experiments done in the past 12 months have employed a variety of techniques and methods. Femoral bone marrow obtained by aspiration from anesthetized donors is administered intravenously to lethally X-irradiated recipients. Such allografted monkeys are then medically supported and observed for the remainder of their lives with frequent measurement of a number of clinical, biochemical, and immunologic parameters. Lymphocyte antigens of the rhesus monkey are studied by standard eosin dye lymphocytotoxicity tests employing rhesus alloantisera obtained from planned immunizations with skin grafts and leukocyte injections. The mixed leukocyte culture technique was adapted for use with monkey leukocytes and provides another method of assessing histocompatibility.

The production of anti-rhesus lymphocyte sera (ALS) has continued with the immunization of rabbits with purified rhesus thoracic duct lymphocytes. The ALS resulting from these immunizations has been tested for activity *in vitro*, and for immunosuppressive properties *in vivo* using rhesus skin graft and GVH systems.

Major Findings: 1) Newer Modes of Immune Suppression of Allograft Rejection: A study of the influence of graft size and x-ray dose upon engraftment of mismatched allogeneic bone marrow was completed. Monkeys received bone marrow grafts varying from 0.5 to 13 x 10^8 per kilogram and x-ray doses from 800 to 1200 rads. Engraftment was seen at all doses of marrow and at all doses of x-ray above 900. Earlier engraftment was seen with larger grafts. A tendency to shorter survival was seen at higher x-ray doses. These data suggest that engraftment after x-ray conditioning is weakly dose dependent in the dose range 0.5 to 13 x 10^8 per kilogram. Three observations are consistent with the hypothesis that no dose of x-ray permits engraftment of mismatched marrow in the monkey without also inducing a level of radiation toxicity so great that even an early and minor graft versus host disease becomes rapidly fatal. These observations were 1) allografted monkeys died earlier than x-ray controls; 2) the histopathology at the early deaths revealed only subtle findings specific for graft versus host disease while the bulk of damage was similar to that seen in ungrafted x-ray controls; 3) doses of x-ray which did not result in early deaths also did not result in engraftment. A consequence of this hypothesis would be that a reduced dose of x-ray in combination with other specific or nonspecific immunosuppression would be needed to secure graft takes in mismatched rhesus donor-recipient combinations.

Because of our earlier interest in the combinations of anti-lymphocyte serum (ALS) and x-ray for transplant immunosuppression, and because of the possibility of stem cell toxicity in some ALS, an *in vitro* marrow culture system was developed for assay of the effect of ALS on marrow growth *in vitro*. By this means stem cell toxicity was shown in preparations of ALS which manifested toxicity to bone marrow grafts *in vivo*. Another preparation of ALS which did not show toxicity *in vivo* also showed no toxicity in the *in vitro* system. Absorption with red cells removed the stem cell toxicity from the ALS as measured in the *in vitro* assay. Retesting of this red cell absorbed ALS *in vivo* is in progress.

2) Rhesus Histocompatibility Antigens: In the rhesus serologically defined histocompatibility system, RhL-A, 11 specificities have been fully analyzed and reported during the current year. Two have not been reported

previously and the others correlate well with antigens previously reported by us and by others.

A monkey has been identified which behaves in mixed lymphocyte culture (MLC) as a homozygote at a putative nonserologically defined locus determining reactivity in MLC. Intensive study of this animal may ultimately permit serological identification of the product of the MLC locus and analysis of the polymorphism of the locus through typing of other animals.

First attempts at identification of alloantisera obtaining antibodies to lymphocyte subpopulation alloantigens (possible "Ia" specificities by analogy to the mouse) have been undertaken. Sera have been found which inhibit binding of immunoglobulin aggregates to the Fc receptor. Similar sera in mouse systems contain anti-Ia antibodies. Identification of possible Ia specificities would permit analysis of the genetics and polymorphism of the system, development of more rapid techniques for typing of Ia specificities, and ultimately perhaps testing in a primate system immunological manipulations utilizing knowledge about Ia antigens which may have possible significance for induction of transplantation tolerance.

3) Development of a Pedigreed Breeding Colony: Several new births in the pedigreed families occurred and plans to add new breeders for the production of 1/2 and 1/4 sibs were initiated.

Significance for Cancer Research and the Program of the Institute: Considerable resources of the NCI have been devoted to clinical bone marrow transplantation in leukemia and aplastic anemia. Many problems have become apparent with increased clinical experience in this area and these cannot often be studied readily in patients. For instance, *in vivo* validation of *in vitro* techniques for definition of bone marrow stem cell toxicity of agents such as ALS can be accomplished only in animal models. Only HL-A matched donors are being used for clinical bone marrow transplantation. This greatly limits the application of this form of therapy, as well over 60% of all potential recipients do not have matched donors. Transplantation of mismatched marrow has almost invariably resulted either in no graft take or fatal GVHR and even in perfectly matched donor-recipient combinations fatal GVHR may occur, although at a lower frequency. Our recent findings with respect to the modification of GVHR with ALS and RhL-A matching provides tools for study of the basic mechanism of GVHR and also may be applicable clinically, particularly in situations where it is necessary to transplant marrow between individuals not perfectly matched at HL-A. An understanding of the pathophysiology of GVHR obtained in this system will be of benefit in planning new methods of intervention in this problem as it occurs in man. Marrow transplantation may have a role in tumor immunotherapy, but understanding of GVHR is certainly a prerequisite to this. This work supports Objective 6, Approach 1.

Proposed Course of Project: A shift in emphasis is planned from nonspecific immunosuppression involving ALS and x-ray to study a specific immunosuppression. As a basis for this in the monkey, studies during the next year will be restricted to elucidation of the RhL-A system of serologically defined antigens, of the aggregate binding inhibition system of possible "Ia" specificities, and other aspects of the genetics of the RhL-A major histocompatibility complex. Additions to the colony through selected matings within the monkey families will be continued.

Honors and Awards: None

Publications:

Neefe, J. R., Balner, H., Barnes, A. D., et al: Progress in Rhesus Histocompatibility Workshop. Tissue Antigens, in press.

Key Words: Bone Marrow Transplantation; Graft-versus-host Reaction: Transplantation Antigens

Serial No. NCI Z01-CBD 5016(c)-05 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular Immunity II

Previous Serial Number: Same

Principal Investigator: John R. Wunderlich, M.D.

Other Investigators: Dean L. Mann, M.D., William D. Terry, M.D.

Contractor: Litton-Bionetics Research Laboratory

Contract N01-CB-02207

This progress report represents only a portion of work supported by Contract N01-CB-02207

Cooperating Units: R. Michael Blaese, M.D., Metabolism Branch, DCBD, NCI; John Potter, M.D., Georgetown University

Man Years:

Total:	3 1/2
Professional:	1/2
Others:	3

Project Description:

Objectives: This project has been directed primarily at understanding cell-mediated immune cytotoxicity in humans. The long-range goals are: 1) to identify human cells capable of destroying foreign cells by cytotoxic, cell-mediated immune reactions, 2) to characterize factors which influence development of cellular immune effector cells, 3) to characterize mechanisms of foreign cell destruction by effector cells, and 4) to define changes occurring in the cellular immune cytotoxic responses which accompany immune deficiency diseases, malignancies, transplanted organs, autoimmune states, and iatrogenic immune suppression. During the last year we have concentrated on characterizing factors which influence development of cytotoxic cells (Goal #2) and on cytotoxicity tests for melanoma patients associated with an NCI immunotherapy protocol.

Methods Employed: 1) Human Direct, Cell-Mediated Immune Cytotoxicity This cytotoxic pathway is measured by the release of ^{51}Cr from labeled target cells incubated with immune cells for 4 hours. Details of the assay utilizing human cells have been described elsewhere (Wunderlich, et al., Transplantation 13:31, 1972). Target cells are prepared from peripheral blood, pathological fluid exudates, tissue culture lines, and biopsy specimens of lymph nodes and solid tumors. Solid tumor samples and cells adhering to tissue culture surfaces are mechanically separated. Aggregated cells are allowed to settle. Red cells are then removed by ammonium chloride Tris lysis. The resulting cell suspension is labeled with ^{51}Cr. Normal and immune lymphoid-attacking cells are prepared primarily from human peripheral blood. Heparinized peripheral blood cells are purified by nylon column fractionization or by centrifugation with Ficol-hypaque. Studies of multiply transfused humans have shown that cytotoxicity in this assay is specific for direct cell-mediated reactions and independent of conventional complement, humoral antibody, and nonspecific toxins.

2) Human Lymphocyte Dependent Antibody (LDA) Mediated Cytotoxicity. This cytotoxic pathway is also measured by a chromium release assay. After ^{51}Cr labeling, target cells are washed by centrifugation and then incubated for 1/2 hour with heat inactivated serum or plasma containing LDA. Incubation mixtures include carrageenan, a C1 attachment inhibitor. Target cells are again washed and then incubated with nonsensitized attacking lymphoid cells for 4 hours.

3) In Vitro Cytotoxicity Testing for Melanoma Patients on the NCI Cooperative Melanoma Immunotherapy Protocol. Peripheral blood samples from patients in the Immunotherapy Protocol are being evaluated for direct cell-mediated cytotoxicity (see above), lymphocyte-dependent antibody (see above), and complement-dependent antibody. The vaccine cell line used for clinical immunization is also being used as a target cell in these assays. The complement assay is a two-stage, ^{51}Cr-microcytotoxicity test utilizing rabbit complement.

Major Findings: (1) We have further investigated our previous finding that activation of human lymphoid cells nonspecifically increases their effector cell activity in the antibody dependent cellular cytotoxicity (ADCC). One of the mechanisms for increasing effector cell activity appears to involve release of factors from activated cells, which secondarily augment effector cell activity; thus, cell free media from mitogen activated lymphoid cells increase effector cell activity of normal lymphoid cells by a mechanism which does not involve carry-over of the original mitogen. We have also found that effector cell activity in activated cells is found predominantly in small cells, indicating that effector cells are not dividing or that cytotoxic activity is limited to a certain portion of the cell cycle. Effector cell activity also distributes almost equally between E rosetting and nonrosetting lymphoid cells; E rosetting cells contain less than 1% contamination of B cells, monocytes, polymorphonuclear cells, and platelets, and thus are considerably enriched for T lymphocytes. The implication of these findings is that T lymphocytes may be able to serve as effector cells in ADCC.

(2) In vitro cytotoxicity tests have been carried out in order to monitor the response of melanoma patients to the immunotherapy vaccine. Last year 140 patient samples were screened for direct cell-mediated cytotoxicity, lymphocyte-dependent antibody, and complement-dependent antibody against the vaccine cell line. 140 normal donor samples were screened for direct cell-mediated cytotoxicity against this line. The principal findings have been as follows: Lymphoid cells from both normal and patient donors have been cytotoxic for the vaccine cell line. Immunotherapy involving administration of either low or high doses of vaccine has failed to increase cellular immune activity. Complement-dependent antibody against the vaccine cell line has significantly increased following treatment with high dose, but not with low dose vaccine. Lymphocyte-dependent antibody activity has not been significantly increased by low or high dose vaccine administration.

Significance for Cancer Research and the Program of the Institute: (1) Antibody-dependent cellular cytotoxicity provides a mechanism by which normal lymphoid cells can be utilized for target cell destruction. This is important for tumor rejection, since many of the host lymphoid cells attracted to reaction sites may be nonsensitized cells. Not all host lymphoid cells, however, appear to be capable of mediating antibody-dependent cellular cytotoxicity. Thus it is important to identify those host cells which can operate as effector cells if we hope to assess the antitumor properties of host cells which infiltrate the tumor. Not only is the class of lymphoid cells important (T versus B lymphocytes, etc.), but also the state of cell activation. Activation of cells appears to stimulate release of factors which further increase effector cell activity of neighboring host lymphoid cells. This snowballing effect can greatly increase effector cell activity at the target cell site.

(2) In vitro cytotoxicity tests are being used to help assess the effectiveness of immunotherapy for melanoma patients. Although these tests may detect patient responsiveness to allogeneic transplantation antigens, they nevertheless serve the useful purpose of detecting nonresponsive patients or an ineffective vaccine. This work supports Objective 6, Approach 1.

Proposed Course of Project: The project will concentrate on continued evaluation of patients on the melanoma immunotherapy protocol by assessing humoral and cellular cytotoxic immune responses to the vaccine cell line.

Honors and Awards: None

Publications:

Wunderlich, J., Rosenberg, E., Connolly, J. M., and Parks, J.: Characteristics of a cytotoxic human lymphocyte dependent antibody. J. Natl. Cancer Inst., in press.

Rosenberg, E. B., Rogentine, G. N. Jr., Connolly, J., and Wunderlich, J. R.: Anti-HL-A7 lymphocyte dependent antibody. Tissue Antigens, in press.

Connolly, J. M., Schwartz, R. H., Handwerger, B. S., and Wunderlich, J. R.: Augmented antibody-dependent cell-mediated cytotoxicity following sensitization or nonspecific stimulation of human effector cells. Transplantation, in press.

Key Words: Antibody-dependent cellular cytotoxicity; in vitro cytotoxicity tests for melanoma immunotherapy protocol.

Serial No. NCI Z01 CBD 5017(c)-04 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of the Biological Significance of Cell Surface Antigens with Emphasis on Antigens Revealed by Neuraminidase Treatment and on Histocompatibility Antigens

Previous Serial Number: Same

Principal Investigator: G. Nicholas Rogentine, Jr., M.D.

Other Investigators: Enser W. Cole, III, M.D.

Cooperating Units: Warren Strober, M.D., NCI, Robert Trapani, Ph.D., Microbiological Associates, Inc., Paul Chretien, M.D., NCI, John Whitaker, M.D., University of Tennessee, Memphis, William Bunney, M.D. and Elliot Gershon, M.D., NIMH.

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: Definition of the natural biological significance of certain human cell surface antigens is our objective. We have studied two groups of antigens. The first is an antigen or set of closely related antigens revealed after neuraminidase treatment of intact cells. The second group is the well known HL-A antigen system. Nothing as yet is known of the biological significance of the first group of antigens. The latter group has importance for organ transplantation, but our emphasis is upon the relationship of HL-A antigens to the natural function of the human organism.

We are approaching the significance of the neuraminidase revealed antigen(s) by studying the chemical nature of the antigen, its distribution on different cells, its naturally occurring autoantibody, and the *in vivo* distribution of neuraminidase treated cells.

We are seeking the significance of HL-A antigens by investigating their frequency disturbances in different diseases. If such disturbances are found, and many have been, we can design further studies on the role of the antigen in the pathophysiology of disease.

Methods Employed: Cell surface antigens are assayed by standard ^{51}Cr release or dye exclusion cytotoxicity tests. Cells, usually lymphocytes, are either untreated or incubated with Vibrio cholera neuraminidase under conditions which do not effect cell viability but which remove most of the cell surface sialic acid. Estimates of antigen content of different cells are achieved by quantitative inhibition of antisera. HL-A typing is being conducted on contract by Dr. Robert J. Trapani, Microbiological Associates, Inc.

Major Findings: In the past year we have studied two aspects of the neuraminidase revealed antigen problem. The first study was an analysis of the serum antibody levels in normals as well as in patients with newly diagnosed malignancies and in patients with immunodeficiency disease who are known to be in a high risk category for subsequent development of malignancy. We had hypothesized that the natural antibody to the neuraminidase revealed antigen was part of an immunosurveillance system. If this were true, patients with newly diagnosed malignancies and immunodeficiency patients prone to develop malignancy should have low antibody levels. We studied antibody levels in 60 patients with lung, breast and colo-rectal cancer, 20 sarcoma patients, and 20 acute lymphocytic leukemia patients. The distribution of antibody levels in these patients did not differ from age matched normals. In general both older cancer patients and older normals had lower antibody levels. Patients with hypogammaglobulinemia and Wiskott-Aldrich syndrome had negligible serum levels as would be expected since both diseases are associated with depressed antibody synthesis. We were able to study three patients with ataxia telangectasia who subsequently developed cancer. Only one of the three precancer serums had low level natural antibody. We concluded that low levels of this antibody did not predispose to carcinogenesis, nor did high levels prevent the development of cancer.

The second aspect of this problem concerns the fate of ^{51}Cr labelled, neuraminidase treated, autologous lymphocytes. We wished to test the hypothesis that the natural antibody functioned to remove damaged (neuraminidase treated) cells *in vivo*. Since this experiment is difficult in humans, we chose to use a mouse model system. The C3H strain has high level antibody to its own neuraminidase treated cells. The C57Bl/6 has no (or very low level) antibody. We compared the fate of intravenously injected ^{51}Cr labelled, neuraminidase treated autologous lymphocytes in both strains. While there was a slightly higher amount of splenic uptake at 24 hours in the antibody negative strain, C57Bl/6, incubation of C57Bl/6 neuraminidase treated cells with known antibody containing C3H serum did not modify the distribution of these cells. We were unable to detect any important differences in the *in vivo* fate of transfused neuraminidase treated cells in the high and low-or-absent antibody strains. We concluded that the antibody did not have a major function in removing neuraminidase treated cells from the blood.

Studies of the association of HL-A antigens and disease continued in several areas. Of major importance was our finding that lung cancer patients with HL-A5 and/or W19 had a significantly better chance (60%) of surviving disease free for one year posttherapy than did lung cancer patients not having these antigens (15%). The series is not large and needs confirmation with a larger prospective study. However, if true, this finding is in accord with our similar observation in acute lymphocytic leukemia and suggests a more general phenomenon in cancer which may be of prognostic and mechanistic value.

We also completed a study of a large number of patients with cancer of the cervix, head and neck and esophagus which failed to show any very significant associations with HL-A antigens. The specific question of survival and HL-A type was not addressed in this study.

We continued to type melanoma patients and to assay post immunization (with a killed allogeneic melanoma cell vaccine) sera for anti-HL-A activity.

We continued to study patients with gluten-sensitive enteropathy and dermatitis herpetiformis and we have collaborated with Dr. Strober on some in vitro experiments attempting to block with anti-HL-A8 serum the effects of gluten peptides on the synthesis of alkaline phosphatase in intestinal mucosal biopsies of these patients.

We are assaying HL-A antigens in patients with well documented acute schizophrenia, since there is some evidence for disordered immunity in this disease. In our small series there is a preponderance, just barely significant, of HL-A2. More patients are needed.

Finally we are assaying HL-A antigens in patients and family members with affective disorders, eg. manic-depressive psychoses, since it is known that forms of this disease are genetic. We are looking for linkage between HL-A and this disease.

Significance for Cancer Research and the Program of the Institute: Cell surface molecules are involved in important recognition phenomena controlling normal growth and development. Malignancies arise when this recognition mechanism fails. Thus the study of these molecules is of critical importance to understanding normal growth and development and malignancy. Fortunately many cell membrane structures are antigenic, allowing identification by standard immunologic techniques. It is our purpose to study selected plasma membrane antigens and their respective antibodies.

Neuraminidase treated tumor vaccines are being widely used in human and animal tumor immunotherapy. An understanding of the immunological nature of the cell surface antigenic change following neuraminidase treatment is essential to the rational application of this promising mode of therapy. Moreover, the study of the natural autoantibodies to the neoantigen may be of great importance in understanding natural cell death or mechanisms of immune surveillance.

The cell surface histocompatibility antigens are a product of an extremely polymorphic genetic system which must have important survival value for the individual irrespective of its role in allograft rejection. For example, the possession of a given HL-A specificity may confer, by mechanisms unknown, susceptibility (or resistance) to a particular disease. Therefore, one rational approach to assessing the natural biological significance of HL-A antigen is the study of the association of disease with these antigens. If such associations are found, they have double significance. Closer medical observation of individuals possessing disease-associated HL-A antigens may result in earlier diagnosis and better control of the disease. In addition such knowledge may help us design experiments to probe the disease's basic pathophysiology. This work supports Objective 6, Approaches 1 and 4.

Proposed Course of Project: Further studies of the antigen revealed by neuraminidase treatment of human lymphocytes will focus on the function of the antibody, the tissue distribution of the antigen and the biological significance of this phenomenon. A variety of patients with diseases, especially malignancies and disorders of immunity, will be typed for HL-A antigens. In particular, we plan large scale prospective studies of the association of HL-A antigens with survival in acute leukemia and lung cancer. Correlations will be sought and experiments designed to probe the pathogenesis of the disease will be attempted.

Honors and Awards: None

Publications:

Graff, K.S., Simon, R.M., Yankee, E.A., DeVita, V.T., and Rogentine, G.N.: HL-A antigens in Hodgkin's disease. J. Nat. Cancer Inst. 52: 1087-1090, 1974.

Everson, L.K., Plocinik, B., and Rogentine, G.N., Jr.: HL-A expression on the G_1, S and G_2 cell cycle stages of human lymphoid cells. J. Nat. Cancer Inst. 53: 913-920, 1974.

Gebhard, R.I., Falchuk, A.M., Katz, S.I., Sessoms, C., Rogentine, G.N. and Strober, W.: Dermatitis herpetiformis: Immunologic concomitants of small intestinal disease and relationship to histocompatibility antigen HL-A8. J. Clin. Invest. 54: 98-103, 1974.

Rogentine, G.N., Plocinik, B.A.: Carbohydrate inhibition studies of the naturally occurring human antibody to neuraminidase treated human lymphocytes. J. Immunol. 113: 848-858, 1974.

Dellon, A., Rogentine, G.N., and Chretien, P.: Prolonged survival in bronchogenic carcinoma associated with HL-A antigens W-19 and HL-A5. A preliminary report. J. Nat. Cancer Inst., in press, 1975.

Key Words: Cell Surface Antigens; Neuraminidase; Histocompatibility; HL-A.

Serial No. NCI Z01 CBD 5018(c)-04 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Function of Lymphocyte Surface Receptors and the Mechanism of Lymphocyte Killing of Target Cells

Previous Serial Number: NCI 5018(c)

Principal Investigator: Pierre A. Henkart, Ph.D.

Other Investigators: John L. Ryan, M.D., Ph.D., Schmuel Ben-Sasson, Ph.D.
Elaine L. Alexander, M.D., Ph.D.

Man Years:

Total:	4 1/2
Professional:	3 1/2
Other:	1

Project Description:

Objectives: To define the mechanism by which lymphocytes destroy foreign cells. To dissect the killing process into discrete steps which can be studied independently. To define the metabolic morphological and functional changes in lymphocytes associated with their binding to a target via their Fc receptor.

Methods Employed: Thus far, we have begun studying the mechanism of antibody dependent lymphocyte killing because the Fc receptor on the attacker cell surface serves as a relatively well understood means of recognition of the target cell. The attachment phase of the killing reaction was modeled by preparing plastic surfaces coated with absorbed fetal calf serum, TNP modified, and then incubated with anti-TNP antibodies. When human lymphocytes settle on such surfaces, the Fc receptor bearing cells adhere to it and the nonadherent lymphocytes can be removed. This enables the observation of the adherent cells and effects a separation of Fc receptor bearing cells from others. The adherent cells can then be removed from the surface, and the morphology and surface receptors of both fractions have been studied by scanning and transmission EM as well as phase and fluorescence microscopy.

The cytolytic phase of the killing reaction was studied by allowing human lymphocytes to settle on antibody coated lipid bilayer membranes as a model for the target cell membrane. DNP-phosphatidyl ethanolamine was incorporated into a horizontal lipid bilayer and the electrical conductance (reflecting ionic permeability) was measured by techniques previously developed for bilayers.

The effect of binding antigen-antibody complexes to the Fc receptor on B lymphocyte functions was studied by looking at DNA synthesis (^{3}H thymidine incorporation) with and without stimulation by mitogens.

Major Findings:

1) Antibody coated plastic is a highly useful preparative technique for separating human lymphocytes into T and B cell populations. Surface markers show that the cells which adhere are B cells, bearing the Fc receptor. The preparative yield is approximately 80%. The nonadherent cells are severely depleted of killing activity in the antibody dependent killing reaction, while the released adherent cells show a variable amount of killing activity due to the demonstrable presence of antigen-antibody complexes on their Fc receptors.

2) The morphology of lymphocytes adhering to antibody coated plastic undergoes a rapid and dramatic change from the villous spherical shape in suspension to a flattened "fried egg" appearance with ruffled membranes at the periphery, and thence to the uropod morphology classically associated with the motile lymphocyte. These changes can be blocked by drugs: azide prevents uropod formation while cytochalasin B, vinblastine and decrease of temperature to 0°C prevent both flattening and uropod formation.

3) Lymphocytes induce a conductance increase of several orders of magnitude in antibody coated lipid bilayers, without resulting in breakage. Neither antibody alone nor lymphocytes alone show this effect. The conductance increase is dependent on a membrane potential which is positive on the lymphocyte side with respect to the other side, as is the case with a natural target cell membrane. Small discrete conductance increases are seen in some experiments, leading to the suggestion that lymphocytes are creating ion-conducting channels in the bilayer.

4) The binding of lymphocytes to antibody coated plastic does not trigger an increase in DNA or protein synthesis. However, preliminary results show that such binding does inhibit the mitogenic effect of lipopolysaccharide and Con A, but not that of PHA. Thus the proliferative signals in cells with Fc receptors appear to be suppressed by binding immune complexes to these receptors.

Significance for Cancer Research and the Program of the Institute: Our method for separation of human T and B lymphocytes, a serendipitous result of studying lymphocyte killing provides an important tool for cellular immunology in humans. It is currently being exploited by Dr. Dean Mann in studying B lymphocyte specific alloantigens.

Our experiments are designed to elicit the mechanism of lymphocyte destruction of foreign cells, believed to be the principal process responsible for destruction of grafts and tumors. I believe we have obtained new insights into two phases of this process. It seems plausible that the morphological change we have observed is a necessary part of the lymphocyte killing process. The drug sensitivities are similar to those seen for killing and morphological studies of the killing process have always described the killer lymphocytes as being motile and flattened against the target cell.

The finding that lymphocytes increase the ionic permeability to lipid bilayer targets strongly suggests that this is the basic mechanism responsible for target cell death. Previous experiments by others showed that dying target cells first released small ions, followed later by macromolecules. Taken together, these and our results point to a colloid osmotic lysis of the target cell. Our experiments are the first which show that the primary damage of the lymphocyte on its target cell is to the latter's membrane.

The biological role of the Fc receptor is at present poorly understood. Our studies with mitogens suggest that the Fc receptor plays a regulatory role in lymphocyte proliferative responses. This work supports Objective 3, Approach 5.

Proposed Course of the Project: Studies of lymphocytes adherent to antibody coated plastic are continuing to examine the hypothesis that the shape change is a necessary step for lymphocytes to kill. We plan to examine the effect of drugs which affect cAMP and the cGMP levels, and also to study the cells from patients with CLL, which have Fc receptors but do not kill.

Lipid bilayer studies are continuing to define the ionic selectivity of the permeability increases of the membrane and if possible determine the size of the channel induced by lymphocytes.

Our major thrust in the immediate future is to generalize both of these sets of findings to immune T lymphocyte killing. We will attempt to obtain immune T lymphocytes which kill cells bearing a chemically defined moiety which can be attached to target cells, plastic surfaces, and lipid bilayers.

Honors and Awards: None

Publications:

Henkart, P. A. and Fisher, R. I.: Characterization of the lymphocyte surface receptors for Con A and PHA. J. Immunol. 114: 710-714, 1975.

Key Words: Lymphocyte Killing; Fc receptor.

Serial No. NCI Z01 CBD 5021 (c)-03 I
1. Immunology Branch
2. Transplantation Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Antigens Determined by the Major Histocompatibility Locus

Previous Serial Number: Same

Principal Investigator: David H. Sachs, M.D.

Other Investigators: C. Garrison Fathman, M.D., John E. Ricks, M.D., Ph.D., Howard B. Dickler, M.D.

Cooperating Units: None

Man Years:

Total:	3
Professional:	1 3/4
Other:	1 1/4

Project Description:

Objectives: 1) To produce antibodies against defined regions of the major histocompatibility complex. 2) To characterize the reactions of these antibodies with the cell surface of lymphocytes, and to determine the nature of the cells bearing individual antigens. 3) To attempt to produce anti-idiotypic antibodies against the receptors on these antibodies which detect cell surface histocompatibility antigens.

Methods Employed: Congenic resistant strains of mice differing only at their major histocompatibility loci have been reciprocally immunized in order to produce antibodies of known, restricted specificity. The antibodies have been characterized by assays of complement-mediated lymphocytotoxicity and by fluorescence microscopy using a fluoresceinated rabbit antimouse immunoglobulin as a developing reagent.

The presence of unexpected specificities in these sera reacting with subpopulations of lymphoid cells has led to the development of methods of distinguishing subpopulation reactions. Additivity studies for cytotoxicity reactions and fluorescent staining have been performed with these reagents in combination with reagents of known specificity for B or T subpopulations of lymphoid cells. Also, reactions with fractionated lymphoid cell populations have been performed. T-cell derived tumors have been used for the absorption of the congenic antisera in order to reveal further specificities for antigens expressed preferentially on B cells. In addition, use has been made of congenic resistant animals bearing recombinant H-2 alleles in order to determine the genetic localization of many of the new antigens.

Antibodies against individual antigenic specificities have been isolated by absorption onto lymphoid cells bearing those specificities, and the antibody-coated cells have then been injected back into syngeneic animals in order to immunize against the "receptor" portions of the antibody molecules. Sera have been collected from these animals after multiple immunizations and are being characterized for the presence of antibodies capable of reacting with the appropriate antihistocompatibility antigen receptors of antibodies and cells. Tests include inhibition of cytotoxicity, binding to solid-phase immunoabsorbents, and *in vitro* and *in vivo* blocking of the immune response to these antigens.

Similar protocols are also being applied to antibodies raised in inbred rats differing at multiple histocompatibility loci, and to cross-species systems.

Major Findings: 1) Several of the antisera produced in congenic resistant mice have been shown to contain additional specificities to those expected in these strain combinations. These specificities have been shown to be determined by genes mapping within the Ir region of the murine major histocompatibility locus, and to be expressed preferentially on B cells. Similar antigenic specificities have simultaneously been described by other laboratories, and the name Ia (Ir associated) antigens has been proposed to describe this new class of cell-surface antigens (1). Anti-Ia antibodies have been demonstrated in several H-2 antisera by absorption with T cell tumors (2).

2) The discovery of one of these Ia specificities on the B cells of a congenic recombinant mouse differing from another mouse only in the Ir region has been found to correlate with the previously described "one-way" mixed lymphocyte culture (MLC) reaction in this strain combination. This has led to the hypothesis that these Ia antigens may be associated with or responsible for the MLC reaction. This hypothesis has been confirmed experimentally using genetically defined MLC combinations and separated cells (3).

3) Anti-Ia antibodies have been found to specifically block the Fc receptor of B lymphocytes (4).

4) Anti-idiotypic reactions against the anti-H-2 antibodies have so far been demonstrable only in the presence of anti-allotype reactions and have been extremely weak.

Significance to Cancer Research and the Program of the Institute:
1) The new Ia antigens we have found appear to provide a new cell-surface marker for the B subpopulation of lymphocytes. In addition, the localization of the genes determining these antigens to the Ir region may indicate that these B cell surface structures are involved in the T cell-B cell collaboration necessary for many immune responses. If so, these antigens may provide markers for dissecting the mechanism of that collaboration.

2) The specificity of the receptor for histocompatibility antigens should reside in the variable portion of the heavy and light chains of the relevant antibody molecules. Thus, anti-idiotypic antibodies against such receptors might be expected to distinguish those cells capable of reacting against individual histocompatibility antigens. Such antibodies, if obtained, could thus provide a new approach to modification of the immune response to cell surface antigens. This work supports Objective 3, Approach 5.

Proposed Course of Project: 1) Other antisera raised between congenic resistant strains of mice differing by known regions of the major histocompatibility complex will be screened for additional Ia specificities. The cellular distribution of these specificities will be determined (i.e., T cells vs B cells), and lymphoid tumors of known cellular origin will be used to absorb differential activities.

2) Models for the functional properties of these antigens which may explain their genetic localization to the Ir region will be further explored. These will include MLC reactivities, cellular cooperation models and cellular cytotoxicity models.

3) The relationship between Ia antigens and the Fc receptor will be pursued. Other non-H-2 antigens also associated with the Fc receptor will be investigated, as will the Fc receptor of other cell populations such as macrophages and T cells.

4) Attempts at immunization against the receptors of anti-H-2 antibodies will be continued. Xenogeneic immunization will be attempted.

5) Extensive testing as outlined above will be required in order to determine whether or not anti-idiotypic antibodies have been produced by any of our immunization protocols. If we can demonstrate the presence of such antibodies serologically, we will then attempt to purify them by selective immunoabsorption on columns bearing the appropriate immunoglobulins. The effects of these antibodies on in vivo cellular and humoral responses to tissue grafts will then be tested.

Honors and Awards: None

Publications:

1. Shreffler, D., David, C., Gotze, D., Klein, J., McDevitt, H. and Sachs, D.: Genetic nomenclature for new lymphocyte antigens controlled by the Ir region of the H-2 complex. Immunogenetics 1: 189-190, 1974.

2. Sachs, D. H. and Cone, J. L.: Ir-associated murine alloantigens: Demonstration of multiple Ia specificities in H-2 alloantisera following selective absorptions. J. Immunol. 114: 165-169, 1975.

3. Fathman, C. G., Handwerger, B. S. and Sachs, D. H.: Evidence for a role of Ir associated B cell alloantigens in MLC stimulation. J. Exp. Med. 140: 853-858, 1974.

4. Dickler, H. B. and Sachs, D. H.: Evidence for identity or close association of the Fc receptor of B lymphocytes and alloantigens determined by the Ir region of the H-2 complex. J. Exp. Med. 140: 779-796, 1974.

5. Sachs, D. H., Fathman, C. G., Cone, J. L. and Dickler, H. B.: Detection of antibodies to Ia antigens in H-2 alloantisera. Transplant. Proc., in press.

6. Sachs, D. H., Cullen, S. E. and David, C. S.: Ir-associated murine alloantigens: Serologic and chemical definition of Ia specificities associated with the H-2^b haplotype. Transplantation, in press.

7. Amerding, D., Sachs, D. H. and Katz, D. H.: Activation of T and B lymphocytes *in vitro* III. Presence of Ia determinants on allogeneic effect factor. J. Exp. Med. 140: 1717-1722, 1974.

8. Sachs, D. H. and Dickler, H. B.: The possible role of I region determined cell surface molecules in the regulation of immune responses. Transplant. Rev. 23: in press.

Key Words: Antigens, major histocompatibility locus, anti-idiotypic antibodies, cell surface antigens, Ia antigens, T cell tumors, Fc receptor, congenic resistant strains of mice.

Serial No. NCI Z01-CBD 5022(c)-03 I
1. Immunology Branch
2. Transplantation Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 31, 1975

Project Title: Genetics of the Idiotype of a Human γA Myeloma Protein

Previous Serial Number: Same

Principal Investigator: John E. Ricks, M.D. and David H. Sachs, M.D.

Other Investigators: William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: 1) To determine where on the immunoglobulin molecule the idiotypic antigenic determinants measured in the previously described radioimmunoassay reside. 2) To determine whether a human myeloma protein contains restricted allotypes and/or idiotypes which are shared with, and can be detected in the serum of, related individuals. 3) To assess the use of myeloma idiotype as a TSTA (Tumor Specific Transplantation Antigen) for following the clinical course of multiple myeloma.

Methods Employed: The IgA myeloma protein was purified by a combination of removal of cuglobulin, Sephadex and anion exchange chromatography. Goat antiserum to the myeloma protein was absorbed with normal human plasma or parental serum and then purified by affinity chromatography. A radioimmunoassay (RIA) has been developed and is capable of measuring about 10 ng/ml of idiotype. The patient's clinical course has been followed and idiotype levels determined by the RIA for 2 years.

Major Findings: 1) No idiotype activity could be found in the sera of 47 other patients with IgA myeloma, nor could any be detected in the patient's parents or 4 siblings. 2) The patient continues in clinical remission, but low idiotype levels are still detectable in his serum.

Significance for Cancer Research and the Program of the Institute: 1) The patient's young age, excellent clinical response, and availability of both parents and 4 siblings present a unique opportunity to study the genetics of idiotype/restricted allotype inheritance in man. 2) The myeloma idiotype provides a marker for disease activity and is being closely followed as an index of the "natural history" of treated myeloma. 3) If the myeloma idiotype can be shown to be present on the surface of this patient's malignant plasma cells, the effectiveness of direct (cytotoxic) and indirect (anti-idiotype antibody coupled to chemotherapeutic agents) specific antitumor therapy can be assessed in vitro. This work supports Objective 4, Approach 2.

Proposed Course of Project: Further characterization of the genetics of this patient's idiotype and restricted allotypes will be carried out by multiple specific absorptions on affinity chromatography columns. Attempts at in vitro specific tumor cell killing will be made as outlined above. The RIA will continue to be used as a tool to follow the course of the patient's disease.

Honors and Awards: None

Publications: None

Key Words: Idiotype; human γA myeloma; radioimmunoassay; tumor specific transplantation antigen

Serial No. NCI Z01-CBD 5023(c)-03 I
1. Immunology Branch
2. Transplantation Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Transplantation Antigens of Swine

Previous Serial Number: Same

Principal Investigator: David H. Sachs, M.D.

Other Investigators: None

Cooperating Units: Surgery Branch, NCI; NIH Animal Center, Poolesville, Md.

Man Years:

Total:	3/8 year
Professional:	1/8 year
Others:	1/4 year

Project Description:

Objectives: To develop at least two strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (SL-A). The animals will subsequently be used for *in vivo* experiments in organ and tissue transplantation and as a source of large numbers of cells from which cell surface antigens can be isolated, purified, and characterized chemically.

Methods Employed: Unlike classical inbreeding schemes which require about twenty generations to produce homozygosity, the approach being used with these swine involves selective breeding on the basis of histocompatibility typing of parents and offspring.

Four males and four females were purchased from commercial sources, and mating pairs were selected to be as varied as possible in order to assure the selection of different histocompatibility genes. Skin grafts were exchanged between the members of each pair and sera were obtained from the animals two weeks after the rejection of the grafts. The sera were tested for cytotoxic antibodies by lymphocytotoxic typing, and pairs which produced strong cytotoxic antibodies were bred.

Offspring from each breeding were tested serologically to determine which histocompatibility antigens had been inherited from the parents, and offspring which could be shown to possess the same antigen combinations were selected for further breeding. This process has now been repeated for four consecutive generations. In addition, mixed lymphocyte culture (MLC) assays have been carried out in parallel to serologic testing of offspring.

Major Findings: 1) Naturally occurring anti-red blood cell antibodies of swine are cytotoxic to lymphocytes (unlike their human counterparts) and must be absorbed from sera with packed red cells before leukocyte typing is performed.

2) Skin grafts between appropriate third and fourth generation animals were rejected in 8-14 days, and the rejection was accompanied by the production of high-titered lymphocytotoxic antibodies. Boosts with lymphocytes have been carried out in several related combinations and have yielded large quantities of high-titered antiserum.

3) Breeding of further generations has continued to be successful.

4) Typing of the fourth generation offspring yielded three breeding pairs of animals homozygous for serologically determined transplantation antigens. We therefore now have the capacity to breed three different herds of swine, each homozygous for a different SL-A antigen.

5) MLC assays of parents and offsprings have continued to show a close correlation between this parameter of histocompatibility and the serologically determined transplantation antigens. Since this type of correlation has previously been observed in studies of man (HL-A), monkey (RhL-A), mouse (H-2), rat (AgB) and dog (DL-A), this finding indicates that we are selectively breeding for homozygosity at a major histocompatibility complex (MHC) homologous to that of other mammalian species.

Significance for Cancer Research and the Program of the Institute: One of the major problems in the study of cell surface antigens of human beings is lack of control of genetic constitution. The use of mice and rats as experimental models avoids this problem, but creates two new ones: 1) physiologically and anatomically these animals are often so different from human beings as to make comparisons and applications of findings difficult. 2) The size of these rodents makes it extremely difficult to obtain sufficient cells and tissues to permit quantitative chemical characterization of relevant cell surface antigens.

For both of these reasons it would be desirable to have animals of size comparable to human beings, whose genetic constitution with respect to histocompatibility could be controlled. The miniature pig, which attains an adult weight of about 200 lbs, seemed ideal for this purpose, and to date the experimental breeding pattern outlined above appears to be working quite well. This work supports Objective 3, Approach 5.

Proposed Course of Project: The breeding plan and typing will be continued. Lines will be established and separated, so that herds of any required size in each line can be produced.

Experiments in collaboration with the Surgery Branch will be carried out in order to: 1) characterize the immune response to transplantation of skin, kidney, and liver in pigs across defined histocompatibility differences, and 2) to determine the basis of liver allograft tolerance which has previously been reported in pigs.

Studies of the SL-A antigens will be initiated at the biochemical level. These will center first on isolation and purification, and then on chemical and immunologic characterization.

Honors and Awards: None

Publications: None

Key Words: Transplantation antigens; miniature swine, SL-A; histocompatibility genes; skin grafts; lymphocytotoxic typing; MLC; breeding.

Serial No. NCI Z01-CBD-5024 (c)-03 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Antibodies to Cell-Surface Antigens of Leukemic Cells

Previous Serial Number: Same

Principal Investigator: Howard B. Dickler, M.D., David H. Sachs, M.D.

Other Investigators: William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: 1) To develop antisera specific for tumor-related antigens on the surfaces of human chronic lymphocytic leukemia cells. 2) To determine the nature of the antigens detected. 3) To develop similar reagents for leukemic cells of mice to serve as a model for experiments using the anti-human leukemia reagents.

This study supports Objective 6, approach 1.

This project is currently inactive due to commitments to other projects. Work will resume on this project as time permits.

Key Words: Antibodies, Cell-surface antigens, Leukemia cells

Serial No. NCI Z01 CBD 5025(c)-03 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic Control of Antibody Responsiveness to Staphylococcal Nuclease

Previous Serial Number: Same

Principal Investigators: Gene M. Shearer, Ph.D. and David H. Sachs, M.D.

Other Investigators: None

Cooperating Units: Jay Berzofsky, M.D., Laboratory of Cell Biology, NIAMDD, NCI

Man Years:

Total: 1/4
Professional: 1/8
Other: 1/8

Project Description:

Objectives: The objective of this project was to investigate in detail the genetic regulation of immune response to nuclease in mice. This species was chosen since the genome of the mouse is better known than that of other rodents. Staphylococcal nuclease is advantageous as a model immunogen, since: (a) its amino acid sequence and three-dimensional structure have been characterized; (b) the immunogen is a protein and appears to be more immunogenic than synthetic polypeptides; (c) a very sensitive enzymatic assay has been developed for detecting antibodies to nuclease; and (d) the molecule can be cleaved into a number of fragments which are capable of binding specific subpopulations of antibodies (Sachs, et al., J. Immunol. 109: 1300, 1972).

Methods Employed: A number of inbred mouse strains as well as congenic resistant lines (differing only at H-2) were immunized with nuclease and their sera tested for anti-nuclease activity. Antibody activity was measured by determining the ability of a given antiserum to inhibit nuclease hydrolysis of denatured DNA using a quantitative spectrophotometric technique. The antibody activity of sera specific for different determinants within the nuclease molecule was determined by a radioimmune assay using nuclease fragments.

Major Findings: The major findings made thus far are:

1) Strain-dependent differences were detected in the antibody responses of a number of inbred mouse strains to nuclease. SJL/J mice were the highest responders to nuclease, whereas DBA/1 mice were the lowest responders. (DBA/1 x SJL/J)F_1 mice were also high responders.

2) The response potential appears to be H-2 linked to some extent. Thus, mice which elicited high antibody responses were $H\text{-}2^{a,s}$ intermediate responses $H\text{-}2^{d,k}$ and low responders $H\text{-}2^{b,q}$. Confirmation of H-2 linkage was obtained using the congenic lines B10 and B10.A. B10.A ($H\text{-}2^{a}$) mice were high responders, whereas B10 ($H\text{-}2^{b}$) mice were low responders. The immune response controlling responsiveness to nuclease in B10 mice was localized to the Ir region. More specifically, with the use of B10.A (2R), (4R), and (5R) recombinant strains, the gene(s) controlling responsiveness to nuclease was localized to the I-B region, in the same region as the gene controlling responsiveness to IgG myeloma and to lactic dehydrogenase.

3) The observations that a wide variation in the response to nuclease among different $H\text{-}2^{k}$ strains of mice and that SJL/J are high responders whereas DBA/1 are low responders (similar to the non-H-2 linked responses of these two strains to (T,G)-Pro--L) raised the possibility that response potential to nuclease in these two strains might not be H-2 linked. However, H-2 typing of individual (DBA/1 x SJL)F_2 immunized mice revealed that both s/s and q/s mice were high responders whereas only the q/q progeny were low responders.

Among the various high and low responder strains, all appear to be synthesizing antibodies specific for a determinant contained in the 1-126 fragment of nuclease. Without exception, secondary immunization resulted in the production of antibodies specific for fragment 99-149 as well.

Significance for Cancer Research and Program of the Institute: This program is relevant in that it examines the genetic regulation of immune responsiveness to a protein of well defined structure. Although synthetic polypeptides have provided important information concerning response genes, a study of such genes associated with responses to proteins can be more meaningful, since most naturally occurring antigens are complex and since protein antigens are generally better immunogens than are many of the simpler synthetic molecules. It remains to be established whether the immune response gene(s) regulating responses to nuclease (as well as those studied by others) will have any significant bearing on susceptibility to cancer or other diseases. This work supports Objective 2, Approach 5.

Proposed Course of Project: Attention will now be focused on stimulation of various immunological compartments with intact nuclease and nuclease fragments in order to define possible hapten and carrier functions of the original immunogen with respect to immune response gene expression, and to determine whether differences can be detected in antibody affinity between high and low responder strains.

Honors and Awards: None

Publications:

Lozner, E. C., Sachs, D. H., and Shearer, G. M.: Genetic control of the immune response to staphylococcal nuclease I: Ir Nase: Control of the antibody response to nuclease by the Ir region of the mouse H-2 complex. *J. Exp. Med.* 139: 1204-1214, 1974.

Key Words: Genetic Control; Antibody Responsiveness; Genetic Regulation; Immune Response.

Serial No. NCI Z01 CBD 5026(c)-03 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic Control of Cellular Immunity to Staphylococcal Nuclease: Antigen-Induced Stimulation of Protein Synthesis

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: David H. Sachs, M.D.

Cooperating Units: None

Man Years:

Total:	1/32
Professional:	0
Other:	1/32

Project Description:

Objectives: The objectives of this study were to establish whether the genetic control of antibody response to staphylococcal nuclease could be extended to include differential stimulation of high and low responder lymphocytes *in vitro* for specific DNA or protein synthesis, and if so, to determine whether the incubation of monospecific alloantiserum with the lymphocytes would inhibit the DNA or protein synthesis.

Methods Employed: Lymph node cells from high and low responder mice which had been immunized with nuclease were cultured in microtiter plates with nuclease or nuclease fragments in a serum-free culture system. The cultures were pulse labelled for DNA or protein synthesis by incorporation of radioactive thymidine or leucine. Monospecific alloantiserum exhibiting specificity exclusive for H-2 were prepared using congenic B10 and B10.A mice. Other alloantiserum (specific for H-2 and other differences) was prepared in DBA/1 and SJL/J mice. The "blocking" of nuclease-induced lymphocyte stimulation is to be tested by incubating the cells with the antisera prior to culture with nuclease or nuclease fragments.

Major Findings: Staphylococcal nuclease specifically stimulated protein synthesis (but not DNA synthesis) in B10.A and SJL/J high responder mouse strains, without detectably elevating protein synthesis in B10 and DBA/1 low responder mice. This contrasts with the repeated observation that significant specific protein synthesis could not be obtained for synthetic polypeptide immunogens. Stimulation of protein synthesis by nuclease fragments 1-126 and 99-149 have given variable results. Lymph node cells fractionated over nylon columns have given interesting results. Both the adherent (B-cell) and non-adherent (T-cell) fractions gave greater stimulation of protein synthesis with nuclease than did unfractionated cells. Attempts to specifically inhibit nuclease-induced protein synthesis of high responder by monospecific H-2 alloantiserum was unsuccessful, since normal mouse serum inhibited the response to the same degree.

Significance for Cancer Research and the Program of the Institute: These observations indicate that an immunological function other than antibody production is under genetic control in the mouse. This provides an opportunity to investigate the cellular aspects of immune response genes regulation at a level other than antibody production. Furthermore, it permits an analysis of the inhibition of lymphocyte stimulation by both restricted (H-2 differences only) and non-restricted (H-2 and other differences) alloantiserum in the elucidation of a possible Ir-gene product on cell surfaces. This work supports Objective 2, Approach 5.

Proposed Course of Project: Efforts will continue to be made to obtain protein stimulation with nuclease fragments and to correlate the genetic control of responsiveness to this immunogen with one of those fragments.

Honors and Awards: None

Publications: None

Key Words: Genetic Control; Cellular Immunity; DNA or Protein Synthesis.

Serial No. NCI Z01 CBD 5027(c)-03 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Identification of a Genetic Complex in the Mouse Other Than H-2 That Controls Multiple Immunological Functions and Antibody Response to More Than One Immunogen

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: None

Cooperating Units: Edna Mozes, Ph.D., Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel and Gustavo Cudkowicz, M.D., Department of Pathology, State University of New York Medical School, Buffalo, New York

Man Years:

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

Objectives: The H-2 region in the mouse and the analogous major histocompatibility regions in other species have been shown to be genetic regions which control immunological responsiveness to a number of immunogens (McDevitt and Benacerraf, Advanc. Immunol. 11: 31, 1969). Nevertheless, other less obvious genetic complexes which regulate immunological functions for a number of immunogens could also exist. The Ir-3 gene is an example of a non-H-2 linked immune response gene which controls responsiveness to the synthetic polypeptide (T,G)-Pro--L. SJL/J mice are the lowest responders to (T,G)-Pro--L, whereas DBA/1 mice are the lowest responders (Mozes, et al., J. Exp. Med. 130: 493, 1969). The purpose of the present study was to establish whether a genetic region can be identified in the mouse other than H-2 which regulates multiple immunological function and responsiveness to a variety of immunogens.

Methods Employed: The SJL/J and DBA/1 mouse strains as well as F_1, F_2 and backcrosses between these two strains are being studied with respect to their ability to respond to the loop and Pro--L moieties of loop-Pro--L, as well as their ability to reject hamster bone marrow cell grafts. The ability of individual F_2 and backcross animals to respond to the above mentioned antigens and immunological functions are being studied for possible association of responses as well as linkage to allotype.

The animals are being immunized with loop-Pro--L and their sera assayed for anti-Pro--L and loop activity by hemagglutination. Expression of a hybrid histocompatibility gene is being tested by injecting hamster cells into irradiated F_1, F_2 and backcross animals whose response for the above immunological parameters have been previously determined.

Major Findings: The major findings made thus far are: The antibody responses to both the loop peptide of lysozyme and Pro--L are not H-2 linked. There is a striking difference in the ability of DBA/1 and SJL/J mice to reject grafts of hamster bone marrow. Irradiated DBA/1 mice reject hamster hemopoietic cells, whereas hamster marrow grows in SJL/J hosts. Studies in F_2 recipient mice indicate that at least one but not more than two non-H-2 linked genes control the ability of these mice to react to the hamster marrow. It has not yet been established whether this gene or genes is linked to antibody responsiveness to loop or Pro--L.

Significance for Cancer Research and the Program of the Institute: If evidence can be obtained indicating the existence of a genetic region for expression of immune responsiveness other than H-2, then genetic regulation of immune responsiveness would be much more complex than is presently assumed, and another genetic complex of immune response will have been identified. Such an observation would expose an additional gene complex whose function for regulation of immune processes for both the normal and neoplastic tissue recognition could be pursued. It may be significant that the SJL/J mouse strain (which expresses immune gene functions as summarized above) is also a strain which develops viral-associated leukemia (Haran-Ghera and Kötler, Bibl. Haematol. 31: 70, 1968), and that the immune response potential of infected animals is reduced (Shearer, et al., J. Immunol. 110: 736, 1973). This work supports Objective 2, Approach 5.

Proposed Course of Project: Offspring of the F_1, F_2 and backcross mating are being individually studied with respect to their ability to respond to the immunological parameters outlined above. Should an association of two or more parameters be established, attempts will be made to link these immunological function to known murine genetic markers.

Honors and Awards: None

Publications: None

Key Words: Genetic Complex; Other than H-2; Controls Multiple Immunological Functions; H-2 region; Non-H-2 Linked Immune Response; Other Than H-2 Which.

Serial No. NCI Z01 CBD 5028(c)-03 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Separation of Functionally Distinct Immunocompetent Cells by Fractionation over Histamine Columns

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: None

Cooperating Units: Elizabeth Simpson, Vet. M.B., Surgical Sciences, Clinical Research Center, England; Kenneth L. Melmon, M.D., Chairman, Division of Clinical Pharmacology, University of California Medical Center, San Francisco, California; Yacob Weinstein, Ph.D., Postdoctoral Fellow, Division of Clinical Pharmacology, University of California Medical Center, San Francisco, California

Man Years:

Total:	1/16
Professional:	1/16
Other:	0

Project Description:

Objectives: It has been recently reported that fractionation of mouse spleen cells over columns of histamine-rabbit serum albumin-Sepharose results in a depletion of the number of anti-sheep erythrocyte plaque-forming cells (PFC) passing through the columns and in an elevation of the immune response potential of the same cell suspension when transferred into irradiated recipient mice (Shearer, et al., J. Exp. Med. 136: 1302, 1972). The objective of this study was to test the effects of such cell fractionation for other immunological functions, and to elucidate the cellular mechanisms involved.

Methods Employed: Histamine rabbit serum albumin-Sepharose (HRSA-S) and rabbit serum albumin-Sepharose (RSA-S) beads were prepared by coupling histamine to RSA with ECDI, and then binding the proteins to CNBr-activated Sepharose 4B. Cells were incubated with the bead preparations (25 x 10^6 cells/0.6 ml of 25% W/v beads), and then non-adherent cells separated from the adherent population by passing the cell-bead mixture through a column containing a cotton plug.

Cells were eluted from the HRSA-S and RSA-S columns by mechanical means. The various functions of cells were tested in vitro for cell mediated cytotoxicity.

Major Findings: The major findings can be summarized as follows: 1) Most of the cell mediated cytotoxic activity was found in the cells retained by the HRSA-S columns, when tested in vitro. In contrast, most of the cytotoxic precursor cell activity appeared to be in the cells that did not adhere to the HRSA-S columns when the cell mediated immunity was generated in irradiated allogeneic mice. 2) In vivo cell transfers yielded elevated PFC responses in the cell population that did not adhere to the histamine beads. 3) No effect of cell separation was observed on the graft versus host response tested in vivo. 4) The effect of histamine-column separation on the enhancement of antibody response potential was found to be due to T-cells and not B-cells. 5) The possibility that the immunobiological effects of the histamine column was due to fractionation of cells on a charge basis was ruled out since poly-L-lysine columns (similar in charge to histamine) had no effect on either the antibody or cytotoxic responses.

Significance for Cancer Research and the Program of the Institute: These results raise the possibility that certain populations of lymphocytes may express surface receptors for histamine. The fractionation procedures can be utilized for separation of cells with different immunological functions. The differential effects of testing the separated cells in vitro and in vivo raises the possibility that radio resistant cells in the host animals may modify immune responses and/or that in vitro culture systems may permit the survival of selected cell populations. This study suggests that receptors for endogenous hormones may be expressed on lymphoid cells and they may be significant in regulating immune processes. This work supports Objective 2, Approach 5.

Proposed Course of Project: Since the histamine columns had opposite effects on in vivo antibody production to sheep erythrocytes and on in vitro cell mediated immunity to alloantigens, cell mediated and humoral immunity to the same antigenic specificity will be required in order to compare the column effects on in vivo and in vitro aspects of these immune parameters.

Honors and Awards: None

Publications:

Shearer, G. M., Weinstein, Y. and Melmon, K. L.: Enhancement of immune response potential of mouse lymphoid cell fractionated over histamine columns. J. Immunol. 113: 597-607, 1974.

Key Words: Separation; Immunocompetent Cells; Histamine Columns; Cell Fractionation; Cellular Mechanisms Involved.

Serial No. NCI Z01 CBD 5033(c)-03 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunotherapy of Human Cancer

Previous Serial Number: Same

Principal Investigator: William D. Terry, M.D.

Other Investigators: Enser Cole, M.D.

Cooperating Units: Steven Rosenberg, M.D., Surgery Branch, NCI; Vincent DeVita, M.D., Medicine Branch, NCI

Man Years:

Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives: This project is designed to study various approaches to the immunotherapy of human malignancies.

Methods Employed: Patients will be evaluated for immunologic status and treated with a variety of immunotherapeutic maneuvers. They will be monitored for changes in general immune responsiveness, as well as for specific responsiveness to tumor antigens.

Major Findings: The stage III disease study has been terminated since the number of patients originally selected have been entered. The results are summarized in the table:

	Chemotherapy	Immunotherapy
Total Patients	13	16
Partial Regression	1(?)	1(?)
Failed	13	16
Deaths	11	11
Surviving Off Therapy	1	5
Median Survival	4.5+ months	6+ months
Mean Survival	5+ months	6.5+ months

There are no significant differences. Neither therapy alone appears to be particularly promising.

Evaluation of immunologic responses indicated that many patients were not being immunized against the 8072 cell line. Selected stage III patients were immunized off protocol to establish a more effective regimen. It was found that increasing the cell dose to 2 x 10^8 VCN treated cells, and giving it more frequently resulted in a high frequency of immunization against the cell line. This was detected by complement dependent cytolysis of the 8072 cell line when incubated with post-immunization, but not pre-immunization serum. Occasional patients also demonstrated lymphocyte dependent antibody killing of the cell line and direct cell mediated cytotoxicity.

Based on these studies, it has been decided that future use of the vaccine will be only at the higher level.

The stage II disease study has been continued, but relatively few patients have been entered into the study to date. Results are shown in the table:

	Control	Chemotherapy	Immunotherapy
Total Patients	7	6	7
Failed	2	2	3
Deaths	0	1	0
Still on Therapy	5	5	4

This study is being revised and a new protocol will be started soon utilizing the higher dose of VCN treated cells.

Significance for Cancer Research and the Program of the Institute: Immunotherapy studies will explore the clinical effectiveness of manipulating the immune system in patients with cancer, and will also provide new information about the biology of the tumor-host relationship. This work supports Objective 6 Approach 1.

Proposed Course of Project: The stage II protocol will be revised and a major effort will be made to enter patients.

Honors and Awards: None

Publications: None

Key Words: Immunotherapy, Human Malignancies, General Immune Responsiveness, Specific Responsiveness to Tumor Antigens.

Serial No. NCI Z01 CBD 5034 (c)-02 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization and Isolation of Human Lymphocyte Subpopulations

Previous Serial Number: Same

Principal Investigator: Howard B. Dickler, M.D.

Other Investigators: Robert D. Arbeit, M.D., David H. Sachs, M.D., John R. Wunderlich, M.D., William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional	3/4
Other:	1/4

Project Description:

Objectives: To characterize and isolate human lymphocyte subpopulations and evaluate the function of such populations in the immune response in both normals and disease states.

Methods Employed: Lymphocytes from normal humans and patients with various diseases are isolated and, both before and after various procedures, are evaluated for surface Ig and the receptor for complexed antibody (primarily B cell markers) and the formation of spontaneous rosettes with SRBC (T cell marker). Lymphocyte subpopulations are isolated using a fluorescent-activated cell sorter and density flotation centrifugation procedures. Inhibition of binding of Ig complexes to Fc receptors is used to evaluate human alloantigens which may be B cell specific and associated with Fc receptors.

Major Findings: 1) Peripheral blood lymphocytes from normal humans were found to consist of 4 subpopulations: B cells, T cells, cells bearing both B and T cell markers (double marker cells), and cells bearing no markers (null cells). 2) Evaluation of lymphocyte surface markers has allowed separation of patients with variable immunodeficiency and panhypogammaglobulinemia into distinct groups which appear to differ in the nature of their fundamental defect. 3) Patients with chronic lymphocytic leukemia could be separated into T and B cell types, the latter even when surface Ig was not easily detected. 4) Significant enrichment of both B and T cell populations has been obtained using the fluorescent activated cell sorter or density flotation centrifugation. 5) Lymphocytes which adhere to antibody coated tumor cells were identified as primarily B lymphocytes. 6) Pretreatment of lymphocytes with appropriate HL-A antisera inhibits binding of Ig complexes to the Fc receptors of B lymphocytes.

Significance for Cancer Research and the Program of the Institute: Characterization and isolation of lymphocytes subpopulations is of fundamental importance for an understanding of the nature of the immune response.

Characterization of lymphocyte surface markers has provided further insight into the nature of the basic defects in some patients with immunodeficiency.

Identification of alloantigens associated with Fc receptors and primarily expressed on B cells may be important for understanding immunoregulation and interactions between immunocompetent cells.

This work supports Objective 2; Approach 1.

Proposed Course of Project: Isolated subpopulations of lymphoid cells and clonal populations from disease states will be used to develop reagents specific for each subpopulation. Isolated subpopulations will be evaluated for functional capabilities in the immune response, both alone and in combination.

HL-A antisera will be absorbed with T cells and platelets to remove HL-A reactivity in an attempt to demonstrate B cell specific antigens determined by the MHC.

Honors and Awards: None

Publications:

Dickler, H. B., Adkinson, N. F., Fisher, R. I. and Terry, W. D.: Lymphocytes in patients with variable immunodeficiency and panhypogammaglobulinemia: Evaluation of B and T cell surface markers and a proposed classification. J. Clin. Invest. 53: 832-840, 1974.

Aiuti, F., Cerottini, J.-C., Coombs, R. R. A., Cooper, M., Dickler, H. B. et al.: Identification, enumeration, and isolation of B and T lymphocytes from human peripheral blood. Scand. J. Immunol. 3: 521-532, 1974.

Yust, I., Smith, R. W., Dickler, H. B., Wunderlich, J. and Mann, D. L.: Human lymphocyte dependent antibody mediated cytotoxicity: Adherence of lymphocytes to antibody treated target cells. Cellular Immunol., in press.

Key Words: lymphocyte subpopulations, Fc receptor, surface Ig, B cell markers, T cell markers, variable immunodeficiency, chronic lymphocytic leukemia, HL-A antisera

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunochemical Genetic and Functional Analysis of the Lymphocyte Receptor for Complexed Immunoglobulin

Previous Serial Number: Same

Principal Investigator: Howard B. Dickler, M.D.

Other Investigators: Robert D. Arbeit, M.D., David H. Sachs, M.D., John R Wunderlich, M.D., Pierre A. Henkart, Ph.D.

Cooperating Units: None

Man Years:

Total:	2 1/4
Professional:	1 1/2
Other:	3/4

Project Description:

Objectives: (1) To immunochemically characterize the lymphocyte receptor for complexed antibody; (2) to evaluate the genotypic and phenotypic expression of the receptor; (3) to evaluate the role of this receptor in the immune response.

Methods Employed: Heat-aggregated immunoglobulin and/or antigen-antibody complexes are fluorochrome or radioactive isotope labelled, and then allowed to interact with isolated lymphocytes from both man and animals. Binding is assayed by U.V. and phase microscopy, the fluorescence activated cell sorter (FACS), or by isotope counting. The interaction is studied by manipulation of the conditions of interaction and by immunologic and chemical modifications of both lymphocytes and complexes. Genetic studies are carried out utilizing inbred, congenic, and/or recombinant mouse strains, or patients with immunologic abnormalities. Functionally, the effect of Ig complexes on antibody dependent, cell mediated cytolysis and on the humoral immune response (plaque-forming cell assays) are analyzed.

Major Findings: The receptor for complexed immunoglobulin is not dependent on complement, temperature, pH or divalent cations. It is distinct from surface Ig and the C3 receptor, and is a trypsin-resistant protein or glycoprotein. The receptor is specific for the Fc portion of complexed Ig and the site on the Fc involves disulfide bond(s). The receptor is necessary for antibody-dependent cell-mediated cytolysis.

The Fc receptors of murine B lymphocytes are either identical or closely associated with Ia antigens (Ir gene associated). It appears that Fc receptors/Ia antigens are located in patches or clusters on the cell surface. Antigens determined by at least one non-H-2 locus also are associated with the B cell Fc receptor.

T lymphocytes also appear to have Fc receptors but in lower numbers or having less affinity for complexes than those of B cells.

Significance for Cancer Research and the Program of the Institute: The receptor for complexed antibody has been shown to play a key role in antibody-dependent cell-mediated cytotoxicity. This immunologic mechanism may play an important part in host response to tumors. The Fc receptor/Ia antigen patch is hypothesized to be important in regulation of the humoral immune response and collaborations between immunocompetent cells.

This work supports Objectve 2, approach 1.

Proposed Course of Project: (1) Further analysis of the Fc receptor of T lymphocytes for specificity and possible relationships with Ia antigens and other alloantigens. (2) Study of the Fc receptor of macrophages for possible relationships with Ia antigens in order to assess possible similarities between the Fc receptors of different immunocompetent cell populations. (3) Capping studies to further evaluate the Ia-Fc relationship and the relationship between different Ia specificities on B cells. (4) Analysis of the alteration which occurs in Ig when complexed which permits binding to Fc receptors. (5) Analysis of the relationship between both B cell mitogens and T cell factors which activate B cells and the Fc receptor.

Honors and Awards: None

Publications:

Dickler, H. B.: Studies of the human lymphocyte receptor for heat-aggregated or antigen-complexed immunoglobulin. J. Exp. Med. 140: 508-522, 1974.

Sachs, D. H. and Dickler, H. B.: The possible role of I region determined cell surface molecules in the regulation of immune responses. Transplant. Rev., in press.

Key Words: Fc receptor, antigen-antibody complexes, U.V. and phase microscopy, FACS, congenic and/or recombinant mouse strains, antibody dependent, cell-mediated cytolysis.

Serial No. NCI Z01-CBD 5036(c)-02 I
1. Immunology Branch
2. Transplantation Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Relationship Between Ir Genes and Immunoglobulin Variable Region Genes

Previous Serial Number: Same

Principal Investigators: David H. Sachs, M.D. and C. Garrison Fathman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3/4
Professional:	3/4
Other:	0

Project Description:

Objectives: The antibody response to staphylococcal nuclease (NASE) is under genetic control by gene(s) located in the Ir region of the mouse major histocompatibility complex (H-2). Since the antibodies produced to certain regions of this antigen have been previously shown to demonstrate restricted heterogeneity, we wish to determine whether or not the relevant Ir gene determines which variable region immunoglobulin products are produced. If the Ir gene is found to exert quantitative but not qualitative control over idiotypic antibody production, then a search for a distinct "T cell receptor" for nuclease (i.e., other than known IgV regions) must be anticipated.

Methods Employed: 1) Antibodies to NASE have been prepared in groups of mice of a variety of strains (both high and low responders) differing in H-2 type or allotype or both. Certain of these have been purified by affinity chromatography. Inbred Lewis rats have been immunized with the purified anti-NASE antibodies obtained from immune ascites from two different A/J mice after immunization with NASE. Immunoabsorptions with normal immunoglobulins from the same strain have been used in order to determine whether or not antibodies reactive with the variable region (i.e., anti-idiotypic antibodies) have been produced. 2) Anti-idiotypic reactions are being quantitated by the inhibition of antibody-mediated enzyme inhibition. Such reactivities will then be screened against anti-NASE antibody populations from the other strains of mice in order to determine the possible genetic linkage of idiotype to allotype and/or H-2 type. 3) Attempts will be made to block functional recognition of NASE by lymphocytes using appropriate anti-idiotypes. Depending on the genetic linkage that is found, emphasis will be placed on distinguishing T cell from B cell specificity. 4) The possible expression of idiotypic determinants on specific cell surfaces will be examined using fluoresceinated anti-idiotype and the Fluorescence-Activated Cell Sorter (FACS).

Major Findings: By two separate criteria, anti-idiotype antibodies have been obtained. 1) After exhaustive absorption either with whole A/J ascites or repeated passages over affinity chromatography columns to which normal A/J ascites was bound, antibodies remain which react with A/J αNASE but not with C57BL10 congenic antibody of either responder or nonresponder H-2 type. Prior to absorption these rat antibodies contained precipitating antibodies to either type (A/J or C57BL10) of antibody but no precipitating antibodies remained following absorption. 2) The rat anti-A/J αNASE antibodies prior to and following absorption react with the combining site of anti-NASE antibodies as evidenced by their ability to inhibit the anti-NASE antibody inactivation of NASE in enzymatic assay.

Significance for Cancer Research and the Program of the Institute: The development of an anti-idiotypic reagent directed toward antibodies under the control of an H-2 linked Ir gene may provide insight to the mechanism of Ir gene function. It may indicate whether the specificity of T cell recognition of antigens depends on similar variable region gene products to those responsible for B cell or antibody specificity. It may also lead to possible methods of controlling Ir gene expression. This work supports Objective 3, Approach 5.

Proposed Course of Project: This anti-idiotype will now be used to study the possible H-2 or allotypic linkage of the idiotype in several combinations of H-2 or allotype congenic mice. Additional anti-idiotypic antisera will be raised against the anti-NASE antibodies of other strains of mice. The reactions of each will be characterized by inhibition of binding assays or by radioimmunoassays if necessary. Screening studies will then be performed in order to determine the genetic linkage of the idiotypic specificities distinguished.

If linkage of idiotype expression with H-2 type is established, we will move directly to studies of cell surface receptors on T cells and to manipulations of responsiveness by means of pretreatment with the anti-idiotypic reagents.

If linkage to allotype is established, this relationship will be fully characterized, since previous studies of such linkage have not employed antigens under Ir control. An attempt will then be made to immunize with immune T cells rather than antibody in order to determine whether another "idiotype" can be distinguished for the T cell receptor.

Honors and Awards: None

Publications: None

Key Words: Ir genes; immunoglobulin variable region genes; antibody response; staphylococcal nuclease; genetic control; mouse major histocompatibility complex

Serial No. NCI Z01 CBD 5037(c)-02 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell-Mediated Immunity to Carboxy Pyridine Disulfide-Modified Syngeneic Lymphocyte Proteins: Linkage of Responsiveness to the Murine Major Histocompatibility Complex

Previous Serial Number: Same

Principal Investigators: Gene M. Shearer, Ph.D. and Pierre A. Henkart, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/16
Professional:	1/32
Other:	1/32

Project Description:

Objectives: As described in an accompanying report, it has been possible to generate cell mediated immunity to TNP-modified syngeneic lymphoid cell proteins. The ability to respond was found to be linked to the H-2 complex, as were the specificity restrictions between immunogen and target. The objective of the study outlined here was to determine whether modification of cell surface proteins with protein-binding reagents other than trinitrobenzene sulfonic acid would result in changes that would create immunogenic determinants recognized as foreign by syngeneic lymphocytes, and to investigate the genetic and immunologic parameters described in the accompanying report.

Methods Employed: Lymphoid cells from a number of inbred and congenic resistant mouse strains were heavily modified by treatment with carboxy pyridine disulfide (CPDS). These modified cells were used as immunogens and radioactively labelled target cells for immodified syngeneic and allogeneic responding lymphocytes, in primary *in vitro* sensitization for CML reactivity. Effector cells from the CML sensitization were assayed for cytotoxicity on syngeneic ^{51}Cr-labelled targets.

Major Findings: Although preliminary studies were promising on this project subsequent experiments have been unable to verify the initial observations.

Significance for Cancer Research and Program of the Institute: The same possible points of relevance mentioned in the TNP report apply here as well. This work supports Objective 2, Approach 5.

Proposed Course of Project: Terminated.

Honors and Awards: None

Publications: None

Key Words: Cell Mediated Immunity; Syngeneic Lymphocyte Proteins; H-2 Complex.

Serial No. NCI Z01 CBD 5038(c)-02 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell-Mediated Immunity to Trinitrophenol-Modified Syngeneic Lymphocyte Proteins: Linkage of Responsiveness and Specificity of Reactivity to the Murine Major Histocompatibility Complex

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: Anne-Marie Schmitt, Ph.D.; Terry G. Rehn, M.D.; Susan Cullen, Ph.D.

Cooperating Units: None

Man Years:

Total:	2 1/2
Professional:	2
Other:	1/2

Project Description:

Objectives: The purpose of this project was to investigate *in vitro* induction of cell mediated immunity, i.e., cell mediated lympholysis (CML) and mixed lymphocyte reactivity (MLR) of murine lymphocytes to syngeneic and allogeneic lymphoid cells (normal and neoplastic) modified with trinitrophenol (TNP). This study was designed to establish: a) whether the immune reactivity obtained was specific for the haptenic moiety (TNP) or whether it involved reactivity to some TNP-modified cell surface protein(s); b) whether response potential in this model was associated with an immune response (Ir) gene, and if so to determine the linkage pattern; c) how specific the reactivity is *vis a vis* the genetic and cellular components of the induction and effector phases; d) if the immune reaction is composed exclusively of T cells or if B cells are also required; and e) whether antibodies specific for TNP are generated in addition to the cell mediated response during the course of immunization.

Methods Employed: Lymphoid cells from a number of inbred and congenic resistant mouse strains were heavily TNP-lated by treatment with trinitrobenzene sulfonic acid. These modified cells as well as lymphoma cells were used as immunogens and as radioactively-labelled cytotoxic target cells for unmodified syngeneic and allogeneic responding lymphocytes in primary in vitro sensitization for either CML or MLR reactivity. Effector cells from the CML sensitization were assayed for cytotoxicity on the appropriate (identical and nonidentical to genotype of immunizing cell type) ^{51}Cr-labelled targets and tested for anti-TNP antibody by a TNP-coated sheep erythrocyte plaque assay.

Major Findings: The major findings made thus far are:

1) Cell mediated cytotoxicity to TNP-modified syngeneic spleen and tumor cells was obtained. By assaying the sensitized lymphocytes with TNP-modified cells from an unrelated strain, major reactivity to TNP was ruled out. The response appears to be directed either to a modified cellular protein or to the modified protein plus the TNP moiety.

2) Weak but significant mixed lymphocyte reactivity has been obtained using TNP-modified syngeneic spleen cells as the stimulating population.

3) The relevant cell surface proteins modified by TNP to be immunogenic are controlled by the K and D serological regions of the H-2 complex.

4) H-2 linked immune response genes mapping to the left of I-A control response potential to the newly formed antigenic specificities.

Significance for Cancer Research and Program of the Institute: This project is of basic immunological interest in that it describes a new murine major histocompatibility linked immunological phenomenon. The fact that the phenomenon itself involves immunological reactivity to modified self lymphocyte surface proteins raises the possibility that this is a type of autoimmunity which is H-2 linked. This observation could provide an initial model for investigating the immunological effects of autoimmune and viral-modified lymphocyte surface proteins. This model represents the first demonstration of major histocompatibility control of the formation of new cell bound modified self antigens and of the response potential to these new antigens. It has the potential to provide a working model for bifunctional major histocompatibility control of disease susceptibility in those examples of HL-A-associated diseases. This work supports Objective 2, Approach 5.

Proposed Course of Project: Studies will be performed to determine whether the protein(s) modified by TNP are immune response gene products and/or immune response-associated antigens. Attempts will be made to solubilize, isolate, and characterize the modified cell surface proteins. Once the model has been genetically, chemically, and immunologically characterized, attempts will be made to create "foreign immunogens" using syngeneic cells by modification with viruses and autoimmune complexes. Studies are also in progress to determine whether a similar immunological model can be demonstrated using human leukocytes from normal and autoimmune individuals, and to determine whether any correlation exists between the response potential of individuals and HL-A type.

Honors and Awards: None

Publications:

Shearer, G. M.: Cell mediated cytotoxicity to TNP-modified syngeneic lymphocyte protein. Eur. J. Immunol. 4: 527-533, 1974.

Shearer, G. M.: Recognition of chemically modified autologous cells: Importance of the murine major histocompatibility complex. In Rosenthal, A. (ed.): Ninth Leukocyte Culture Conference. New York, Academic Press, In press.

Shearer, G. M.: Major histocompatibility-linked genetic control of murine cell mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Transpl. Proceedings. In press.

Shearer, G. M., Lozner, E. C., Rehn, T. G. and Schmitt-Verhulst, A.-M.: Mixed lymphocyte reactivity and cell mediated lympholysis to trinitrophenyl-modified autologous lymphocytes in C57BL/10 congenic and B10.A recombinant mouse strains. J. Exp. Med. In press.

Key Words: Cell Mediated Immunity; Modified Syngeneic Lymphocyte Proteins; Murine Major Histocompatibility Complex; Cell Mediated Lympholysis; Syngeneic; Allogeneic Lymphoid; Immune Response (Ir) Gene; T Cells.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Fractionation of Lymphoid Cells Using Nylon Wool Columns

Previous Serial Number: Same

Principal Investigator: Richard J. Hodes, M.D.

Other Investigators: Ronald H. Schwartz, Ph.D., M.D., Barry S. Handwerger, M.D.
Gene M. Shearer, Ph.D., William D. Terry, M.D.

Cooperating Units: Laboratory of Immunology, NIAID, and Department of Medicine, School of Medicine, University of Minnesota, Minneapolis, Minnesota

Man Years:

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives: 1) To identify subpopulations of lymphocytes participating in various immunological reactions in order to gain an understanding of the physiology of the immune system. 2) To investigate the nature of hormonal influences on the immune system by identifying their sites of action.

Methods Employed: During the past year our laboratory has made a significant advance in the separation of mouse lymphoid cells. We have helped develop a simple, rapid, inexpensive and efficient technique for separating large numbers of mouse spleen and lymph node cells into two populations, one enriched for thymus-derived or T lymphocytes and the other enriched for bone marrow-derived or B lymphocytes. The procedure is an extension of a technique first described by Julius, Simpson and Herzenberg (Eur. J. Immunol. 3: 645, 1973). It involves passage of cells over nylon wool columns at 37°C in the presence of Eagle's medium containing 5% heat-inactivated fetal calf serum. The B cells preferentially adhere to the columns, whereas the T cells pass through. After washing the T cells through the columns, the B cells are removed by mechanical agitation. Preliminary experiments indicate that this technique can be extended to other species including humans. (1)

Major Findings: 1) Role of lymphoid subpopulations in immune response systems: (a) Synergy in *in vitro* sensitization. It has been demonstrated that two subpopulations of normal mouse spleen cells, separated by nylon column passage, synergize in the generation of cell-mediated cytotoxicity to allogeneic cells *in vitro*. At least one participating cell is a non-T cell (2).

Similar methods have demonstrated synergy between these nylon-separated subpopulations in the generation of cytotoxicity to TNP-modified syngeneic cells, a system which may represent a model of autoimmunity.

(b) Role of peripheral B and T cells as stimulating or sensitizing cells in mixed leukocyte culture (MLC) and in vitro sensitization. Comparison of multiple lymphoid cell populations, including those separated by nylon column passage, for their ability to sensitize or stimulate in MLC has suggested that both peripheral B and T cells are able to carry out this function.

2. Hormonal influences on immune system. By cell purifications involving nylon, glass wool, and Sephadex G10 passages, it has been demonstrated that monocytes, rather than any lymphocyte population appear to be the insulin binding cells in peripheral human blood.

Significance to Cancer Institute and the Program of the Institute: In the investigation of cell-cell interaction in immune response, the methods employed have already added insights into the nature of the allograft response. The generalization of similar findings to modified syngeneic systems suggests that autoimmune-like responses may involve similar interactions. Preliminary data have also shown that a "syngeneic" response to tumor-specific determinants may also involve this interaction. This work supports Objective 6, Approach 1.

Proposed Course of Project: Work investigating the nature of cells which are able to stimulate in MLC is currently being extended to systems of restricte genetic differences. Such studies are intended to better define the nature of genetic and cell surface determinants relevant to cell interaction and recognition.

Honors and Awards: None

Publications:

Handwerger, B. S. and Schwartz, R. H.: Separation of murine lymphoid cells using nylon wool columns: Recovery of the B cell enriched population. Transplantation 18: 544-548, 1974.

Hodes, R. J., Handwerger, B. S. and Terry, W. D.: Synergy between subpopulations of mouse spleen cells in the in vitro generation of cell-mediated cytotoxicity. Evidence for the Involvement of a non-T cells. J. Exp. Med. 140: 1646-1659, 1974.

Key Words: lymphocyte subpopulations, mixed leukocyte culture, T and B lymphocytes.

Serial No. NCI Z01 CBD 5040(c)-02 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Three-Dimensional Structure of Immunoglobulins

Previous Serial Number: Same

Principal Investigator: David M. Segal, Ph.D.

Cooperating Units: David R. Davies, Ph.D., Section on Molecular Structure, LMB, NIAMDD; Eduardo A. Padlan, Ph.D., Section on Molecular Structure, LMB, NIAMDD; Stuart Rudikoff, Ph.D., Immunochemistry Section, LCBGY, DCBD, NCI; Michael Potter, M.D., Immunochemistry Section, LCBGY, DCBD, NCI

Man Years:

Total:	10
Professional:	10
Other:	0

Project Description:

Objectives: To elucidate the stereochemical basis of antibody specificity and diversity, and to show how antigenic recognition leads to a given response in the immune system. To compare structures of several different homologous domains.

Methods Employed: Mouse myeloma proteins with binding activities toward natural antigens were purified by affinity chromatography, and their Fab fragments prepared by digestion with papain. Crystals of some of the Fab fragments were obtained from ammonium sulfate solutions, and two proteins gave crystals suitable for high resolution diffraction studies.

Crystalline protein-heavy atom complexes were prepared and electron density maps were calculated using standard x-ray diffraction techniques. A three-dimensional model of one protein has been constructed using Kendrew wire models and an optical comparator.

Major Findings: The structure of the Fab fragment of McPC603, a phosphorylcholine-binding IgA, has been determined to 3.1A^{o} resolution. The hapten binding site has been located in a cleft formed by the three heavy chain hypervariable regions, and by the first and third hypervariable regions of the light chain. There are strong interactions between the phosphate group of the hapten and the side chain groups of tyr 33 and arg 52, both of the heavy chain. Acidic side groups from both light and heavy chains are in the immediate vicinity of the positively charged choline group. The hapten is in van der Waals' contact with various portions of the hypervariable loops, but occupies only a small portion of the cleft between the variable domains.

Comparisons of variable domains, especially between members of the same class (i.e. Vk with Vk), indicates a strikingly high conservation of structure in most of the domain. Variability in structure lies in loops, which extend out from the N-terminal end of the V domain, and it is these loops which contain the highest degree of chemical variability. The constant parts of the V domains appear to serve as frameworks which direct the variable loops toward continuous, but variable (between V domains) surfaces. From the limited data available, it would appear that antigenic recognition always takes place on these surfaces, and involves the usual type of macromolecular interactions. The structural basis of antigenic recognition most probably lies in the ability of the framework to segregate and be independent of the variable loops. This ensures the formation of a continuous hypervariable surface.

Significance for Cancer Research and the Program of the Institute: A key link in the major vertebrate (and therefore human) defense mechanism, i.e. the immune system, is the molecular recognition of foreign substances. The present study shows how a given protein molecule specifically interacts with a component found in the cell wall of a wide variety of pathogenic organisms. Moreover, we are currently working out the structure of another myeloma protein with specificity toward a different antigen. Taken together these two studies should give a good indication of how the immune system creates diversity on the molecular level, and what kind of interactions are involved in the recognition phenomenon. It is believed that our studies will give rise to a greater sophistication in both the understanding and control of the immune system. This work supports Objective 6, Approaches 3 and 4.

Proposed Course of Project: The x-ray investigation is being continued by my collaborators. This report thus terminates (except for perhaps another publication) my involvement with this particular project.

Honors and Awards: None

Publications:

Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M., and Davies, D. R.: The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site. Proc. Natl. Acad. Sci. 71: 4298-4302, 1974.

Segal, D. M., Padlan, E. A., Cohen, G. H., Silverton, E. W., Davies, D. R., Rudikoff, S., and Potter, M.: The structure of McPC 603 Fab and its hapten complex. In Proceedings of 2nd International Congress of Immunology. Amsterdam, A.S.P. Biological and Medical Press, 1974, in press.

Davies, D. R., Padlan, E. A., and Segal, D. M.: Three-dimensional structures of immunoglobulins. Annual Rev. Biochem., in press.

Davies, D. R., Padlan, E. A., and Segal, D. M.: Immunoglobulin structures at high resolution. In Contemporary Topics in Molecular Immunology, Vol 4, in press.

Padlan, E. A., Segal, D. M., Cohen, G. H., and Davies, D. R.: The three-dimensional structure of the antigen binding site of McPC 603 protein. In Sercarz, E. E., A. R. Williamson, C. F. Cox (eds.): The Immune System. New York, Academic Press, 1974, pp. 7-14.

Key Words: antibody specificity; myeloma proteins; Fab fragments; x-ray diffraction techniques; three-dimensional model; hapten binding; variable domains.

Serial No. NCI Z01 CBD 5041(c)-02 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characteristics of Lymphoid Populations of AKR/J Mice

Previous Serial Number: None

Principal Investigator: Marion M. Zatz, Ph.D.

Other Investigators:

Cooperating Units:

Man Years:

Total:	3/4
Professional:	3/4
Other:	0

Project Description:

Objectives: The objectives of this project are to: 1) define the defect (if any) in the lymphoid system of AKR/J mice which predisposes this strain to its high spontaneous incidence of thymic lymphoma and lymphocytic leukemia; 2) to characterize the leukemic cell populations in an attempt to determine the target cell which becomes transformed by the Gross Murine Leukemia virus.

Methods Employed: AKR/J mice aged 2 months to a year were studied. Lymph nodes, spleen and thymus cells of young (2-3 month), old (6-9 month), preleukemic (thymoma, but no peripheral organ involvement), and leukemic animals were evaluated for their surface expression of the theta (Θ) AKR (Thy 1.1) antigen, and responsiveness to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A). Tissues from both animals bearing spontaneous lymphomas, and from young AKR/J mice injected with leukemic cells, were studied.

Major Findings:

1. Θ Antigen. There appears to be little change in the percent Θ-positive cells, as assessed by ^{51}Cr release, in the lymphoid organs of mice aged 2 months to a year, either normal or preleukemic. However the spleens and lymph nodes of leukemic animals contain an increased proportion of Θ-positive cells; these cells are more susceptible to lysis by a Θ serum and absorb more

activity from such sera, suggesting an increase in the number of surface Θ determinants. This increase may be due to an increased density of Θ and/or to the increased surface area of such cells.

2. Responsiveness to Mitogens. There is a gradual loss with age in the responsiveness of peripheral lymphoid organs to PHA, which parallels that seen in other strains. A disproportionately greater loss of CON A responsiveness is seen which is most marked in preleukemic and leukemic animals. This disproportionate decline in responsiveness to PHA and CON A results in a progressively greater PHA/CON A ratio as animals age and become leukemic. In the leukemic animals, the changes in mitogen responsiveness are striking and distinctly different in peripheral lymphoid tissues and in thymus. While the PHA and CON A responses of leukemic spleen cells are lower than those of age-matched controls, there is as much as a 50-fold increase in the PHA response of thymoma cells, while the CON A response is often below that of controls.

3. Passage Experiments. In order to determine whether the distinct mitogen patterns observed in the thymus and spleens of leukemic AKR/J mice are stable, these cells have been passed into healthy young AKR recipients. Preliminary results suggest that the mitogen responses of recipient spleen and thymus resemble those seen in the spontaneous leukemic animal. The above results suggest the possibilities that 1) mitogen responsiveness is a more sensitive marker than the Θ-antigen for following changes in subpopulations during leukemogenesis in AKR/J mice; 2) the CON A responsive population may be more readily inactivated by MuLV; 3) microenvironmental influences in spleen and thymus affect the state of differentiation of the transformed cell populations.

Significance for Cancer Research and Program of the Institute: The AKR/J mouse strain provides a valuable model for the study of acute lymphocytic leukemias of the T-cell variety; similar leukemias presenting with thymic lymphosarcomas, have been reported in man. It is hoped that these studies will provide some answers to the question of how and why thymic lymphomas arise, and the nature of the cell populations in which they arise. These studies should also provide more fundamental information about the state of differentiation, and functional activity, of transformed leukemic cells. This work supports Objective 3, Approach 2.

Proposed Course of Project: Several avenues of research will be pursued in an attempt to reach the objectives of this project. They are 1) additional passage studies to determine host influences on the growth of leukemia cells, using recipients modified by thymectomy, immunosuppressive agents or genetic constitution. 2) Studies of how leukemic thymocytes affect the mitogen responses of other leukemic and normal cell populations. The metabolism of such cells will also be investigated with respect to their cyclic AMP and GMP levels. 3) Since AKR mice are not known to have any generalized defects of immune responsiveness, studies will be carried out to examine their specific states of tolerance and/or immunity to virally determined antigens. The approach used will be to examine evidence for cell mediated lympholysis between cells of young, old and leukemic animals. The effects of suppressor cells and serum factors in such assays will also be studied.

Honors and Awards: None

Publications: None

Key Words: AKR/J Mice; thymic lymphoma; leukemia; cell populations; transformed; Gross Murine Leukemia virus.

Serial No. NCI Z01 CBD 5042(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Antigen-Induced Changes in Lymphocyte Traffic

Previous Serial Number: None

Principal Investigator: Marion M. Zatz, Ph.D.

Other Investigators: None

Cooperating Units: R. K. Gershon, M.D., Department of Pathology, Yale University School of Medicine, New Haven, Connecticut

Man Years:

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives: The objective of this project is to determine the factors which influence lymphocyte traffic to the lymphoid organs following antigenic challenge. It is known that a variety of antigens, tumors, allografts, adjuvants, and GVH reaction can induce alterations in lymphocyte homing patterns at the site of antigenic stimulation (Zatz, J. Exp. Med. 134:224, 1971). Such exposure to antigen in vivo usually results in increased localization of cells, termed "trapping," in spleens, or lymph nodes, depending upon the route of antigen administration. Since it is known that lymphocyte trapping is a T-cell dependent phenomenon (Zatz and Gershon, J. Immunol. 112:101, 1974), the question of whether trapping is subject to T-cell regulatory mechanisms was asked.

Methods Employed: Recipient animals of different ages were subjected to immunosuppressive treatments such as administration of ATS, cortisone, or x-ray, prior to challenge with antigen. The magnitude of ^{51}Cr labelled lymphocyte migration to recipient lymphoid organs was then quantitated in immunized, tolerized, or control animals.

Major Findings:

1. The mechanism and/or the cell subpopulations responsible for lymphocyte trapping in spleens and lymph nodes are clearly distinct. Treatment of

recipients with anti-thymocyte serum (ATS), cortisone or 850 Rad irradiation, greatly diminished or eradicated lymphocyte trapping in draining lymph nodes following subcutaneous administration of SRBC or KLH. Identical treatment of recipients resulted in no change or enhancement of lymphocyte trapping in spleens following i.v. administration of antigen. These findings were achieved with both high and low doses of ATS.

2. The ATS-mediated enhancement of splenic trapping was detectable only in young (6-8 week old) animals but not in old (5-6 month old) animals. These results suggest that an ATS sensitive population is largely responsible for production of trapping in lymph nodes. In spleens an ATS sensitive population may regulate the magnitude of the lymphocyte trap in young animals, but is lost with aging. A second population, resistant to the above agents, clearly participates in splenic trapping.

3. In addition to the production of increased localization of lymphocytes (trapping) following antigenic challenge, a net decrease (negative trapping) of lymphocyte traffic can also be demonstrated in response to aggregated HGG and during a GVHr. This negative trap so far has been demonstrated only in spleen and is related to the nature, intensity and timing of the antigenic stimulus. Thus the negative trap is associated with a strong antigenic stimulus, and the stronger the stimulus, the earlier the negative trap is observed. Previous induction of tolerance, or presensitization, abrogates induction of a negative trap.

4. The above results strongly suggest that T-cell subpopulations, particularly in the spleen, are capable of bidirectionally augmenting or suppressing the magnitude of lymphocyte trapping following antigenic challenge. This hypothesis was directly tested by admixing subpopulations of thymocytes (cortisone resistant F_1 or irradiated parental) to GVH-inducing parental thymocyte inocula. The resulting level of trapping during the GVH was then investigated. The results show that subpopulations, which themselves are either weak or inactive in producing a trap, can bidirectionally regulate the magnitude of the trap induced by the parental thymocyte inoculum. When the GVH-induced trap was small these cells enhanced the trapping response, but suppressed it when the GVH-induced trap was large.

Significance for Cancer Research and Program of the Institute: This work is of importance in that it examines the effect, in vivo, of antigenic stimulation on the dynamics of lymphocyte traffic. The regulatory mechanisms described above may play an important role in the number of antigen reactive circulating lymphocytes which become exposed to both immunogenic and tolerogenic stimuli at any given time in vivo. The experiments also provide insight into the differential mechanisms and subpopulations which control lymphocyte traffic in different anatomic lymphoid compartments, i.e. lymph node and spleen. This work supports Objective 6, Approach 1.

Proposed Course of Project: Since trapping has been shown to be a feature of all in vivo responses thus far studied, the above findings are of potential significance in determining the effectiveness of immune responses generated by antigenic tumors. Future experiments will examine the question of how regulation of lymphocyte traffic affects the host's response to such tumors.

Honors and Awards: None

Publications: None

Key Words: Antigen-Induced Changes; Lymphocyte Traffic; Tumors; Allografts; Adjuvants; Homing Patterns; "Trapping"; T-cell Dependent Phenomenon.

Serial No. NCI Z01 CBD 5043(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: AKR Leukemic Lines With T and B Cell Characteristics

Previous Serial Number: None

Principal Investigators: Marion M. Zatz, Ph.D. and Robert S. Greenberg, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives: The objective of this project is to investigate and characterize an unusual variant of the AKR murine leukemia line derived from a spontaneous tumor arising in a 14 month old mouse which had been thymectomized at one month of age. This system was selected as a model for a rarely occurring natural variant of AKR leukemia in which the host animal has an atrophic thymus rather than the usual thymoma.

Methods Employed: Two passage lines of AKR tumors were established and continued by intravenous injection of 1×10^5 spleen cells into young AKR hosts. Donor cells were initially derived from tumors arising in 14 month old animals, one of which had been totally thymectomized at one month of age and one of which had been partially thymectomized and had a thymoma in the thymic remnant and was used as a control.

The passage has been presently carried nine passage generations during which cells were studied for various characteristics including theta antigen (by surface immunofluorescence and cytotoxicity, surface immunoglobulin (SIg), *in vitro* synthesis of immunoglobulin, and mitogen responsiveness. Preliminary attempts at study of aggregate receptors, Ia antigens and passages of cell-free (viral) centrifugates from the two lines are in progress or planned.

Major Findings:

1. Theta antigen is present on 90-100% of spleen and lymph node (LN) cells from both thymectomized-derived and thymic remnant-derived passage cell lines, termed lines A and B, respectively. Theta was best demonstrated using CBA-anti AKR anti-theta serum from hyperimmunized animals counterstained with fluorescein-labelled rabbit anti-mouse gamma_2 immunoglobulin.

2. SIg is demonstrable on up to 100% of A spleen cells by the end of each passage. This SIg is of a single class--μ--as demonstrated by specific immunofluorescence, and does not react with anti-alpha chain or anti-gamma chain labelled antibody. Similar results were obtained using polyvalent anti-immunoglobulin.

The B line spleen cells had 0-5% SIg for the first five passages. In subsequent passage higher levels of IgM -- up to 30% -- were noted at the end of the passage. Spontaneous AKR lymphomas are SIg negative.

3. Within each passage of the A line the percentage of SIg-positive spleen cells increases progressively from 0% (around 10 days) to 90-100% (around 24 days) at times when spleens are grossly enlarged (5-10 fold).

4. LN and thymus cells from A animals with 90% SIg-positive spleen cells and gross lymphadenopathy are theta-positive and SIg-negative.

5. Simultaneous staining for SIg and theta antigen reveal significant numbers (more than 50%) doubly marked cells in the spleen.

6. Preliminary studies on immunoglobulin synthesis in vitro by spleen cells from A and B cell lines demonstrate significant μ and k chain production by A cells. Immunoglobulin synthesis by the B line under the same conditions was less than 10% of A.

There is also another smaller unidentified peak running between μ and gamma on the SDS gel of the A line products. Further studies of Ig synthesis are in process.

7. Mitogen studies through the sixth passage revealed that A cells (late in passage, SIg-positive) responded to LPS normally but not to PHA or Con-A. B spleen cells at the same time had about 33% of the control response to PHA and CON-A, but no response to LPS.

Systematic study of mitogen response at various times during passage and among various lymphoid tissues is presently under way.

Significance for Cancer Research and Program of the Institute: The results of these studies bear upon a number of issues of fundamental importance to both cellular immunology and also to viral leukemagenesis:

a) There are very few cell lines which bear clear markers for both T and B cells, although a normal subpopulation with such characteristics has been identified in small numbers. Perhaps this line represents an abnormal proliferation of this population or an arrested phase of normal lymphoid development, implying transitions between T and B cells.

b) Alternatively the SIg on these cells may represent an abnormal amount of the "normal" T cell immunoglobulin, so that this line might be a way to examine "IgT" and the putative T cell antigen receptor.

c) The difference between spleen and LN cells in the expression of SIg within the same animals suggests that this system may be very helpful in studying the problem of "microenvironment" on cell development within the lymphoid system.

d) The ability to isolate SIg from these cells may allow the generation of anti-idiotype antibody in another species, and thus answer the question of whether or not AKR leukemia is a monoclonal disease.

e) Cell-free extracts of these tumors may allow the identification of a second viral type in the murine leukemia virus (MLV) system.

f) Since the A line is in some sense a B cell leukemia, it may provide a system in which to examine Ia antigen and aggregate receptors.

This work supports Objective 4, Approach 1.

Proposed Course of Project:

Specific immediate plans to follow up some of the above suggestions include: More Ig synthesis studies, including mitogen effects; Mitogen studies; Passage studies of specific lymphoid elements (LN, spleen and thymus); *In vitro* culture lines; Aggregate receptor studies; Ia antigen studies; and Interactions among cell populations.

Honors and Awards: None

Publications: None

Key Words: AKR Leukemic Lines; T and B Cell; spontaneous tumor; thymectomized; atrophic thymus; thymoma.

Serial No. NCI Z01-CBD 5044(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epidermal Cells as Targets of Cell-Mediated Immunity *In Vitro*

Previous Serial Number: None

Principal Investigator: David Steinmuller, Ph.D.

Other Investigator: John R. Wunderlich, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: The overall objective was to develop a simple and objective *in vitro* cell-mediated cytotoxic test with (skin) epidermal cells as targets. The plan was to develop the test initially with mouse cells because of the ready availability of uniform, genetically defined (inbred) strains and some "leads" in the literature where mouse epidermal cells were used as targets for antibody and complement mediated cytotoxicity (e.g. Cooper and Lance, *Transplantation* 11:108, 1971, demonstrated the feasibility of using chromium-labeled epidermal cells in such a system). Later, the extension to other species, including man, would be explored. The specific objective was to adapt the rapid chromium-release assay of Canty and Wunderlich (*J. Nat. Cancer Inst.* 45:761, 1970) for use in microtiter trays with freshly explanted and short-term cultured epidermal cells as ^{51}Cr-labeled targets for allogeneic immune lymphoid cells.

Methods Employed: Suspensions of viable, isolated epidermal cells were prepared by modifying the method of Scheid, et al. (*J. Exp. Med.* 135:938, 1972). Pieces of mouse tail skin were incubated at 37° for 1 hour in 0.5% trypsin in a basic salt solution (BSS) minus calcium and magnesium. The skin then was flattened dermal side up in a dry Petri dish, and the loosened dermis gently removed with watchmakers forceps. The exposed epidermal layer was covered immediately with 0.025% DNAase in the above BSS, and the surface rubbed with the end of a heat-polished Pasteur pipet to free the cells. Fresh media with 10% fetal bovine serum (FBS) was added and the conglomerate filtered initially through nylon mesh to remove debris and large clumps; then through washed glass wool (0.25g) to obtain a nearly uniform single-cell suspension of high (e.g. 80%) viability. The epidermal cells then either were labeled with ^{51}Cr (100μCi/1-3 x 10^7 cells) for immediate use as targets, or put into suspension culture in spinner flasks or allowed to adhere in microtiter trays. "Sensitized" effector cells were obtained by (1) skin allografting mice and harvesting spleen cells 10 days later; (2) culturing normal spleen cells with irradiated (2500 R) allogeneic spleen cells (in 2.5 ml well Linbro tissue culture trays) for 5 days; and (3) by a combination of 1 and 2, i.e. culturing spleen cells from 10-day skin allografted mice with irradiated allogeneic spleen cells and harvesting after 5 days. The assays were carried out by adding effector and labeled target cells (in Eagle's medium with 10% heat-inactivated FBS) together to microtiter trays in the case of nonadherent epidermal cells, or by adding the effectors to wells containing prelabeled and washed adherent epidermal cells. The trays were incubated at 37° for 3 hours in a CO_2 incubator; then spun at 2000 rpm for 10 minutes after which 0.1 ml samples of supernates were taken for gamma-counting.

Major Findings: This project was begun 9/1/74. The major findings as of 2/26/75 are: 1) Existing methods for obtaining viable mouse epidermal cells were improved. The initial yield after trypsinization averages about 7 x 10^6 viable cells per donor tail. Glass wool filtration reduces this yield by about 60%, but results in a nearly uniform suspension of single Malpighian cells of 70-80% viability, as measured by trypan blue dye exclusion.

2) Epidermal cells incorporate about 5 times as much ^{51}Cr as lymphoid cells under identical conditions. The percentage spontaneous release of chromium with time is comparable for fresh epidermal cells and lymphoid cells, usually less than 15% in 3 hours. The percentage release is 2-3 times higher for short-term cultured epidermal cells, however.

3) Significant cell-mediated cytotoxicity in an H-2 different allogeneic strain combination, C57BL/6 anti A, was obtained reproducibly with both freshly explanted and short-term cultured epidermal cells as targets. The best results, in terms of specific percentage lysis, were achieved with the former, probably reflecting the relatively higher "background" (spontaneous chromium release in the presence of normal allogeneic lymphoid cells) with the latter. Effector cells were produced by both in vivo and in vitro sensitization, and the best results were achieved by combining both modes, i.e. by "boosting" in vivo "primed" cells by antigenic exposure in vitro. With these effectors and 5 x 10^4 labeled fresh epidermal cells, cytotoxicity was effector cell dose-dependent over a range of immune (I) to target (T) cell ratios of 1:1 to 64:1, resulting in over 70% specific lysis at the highest I:T ratio.

Significance for Cancer Research and Program of the Institute: Although epidermal cells previously have been used as targets of antibody and complement, there are no reports of their use in a cell-mediated cytotoxic test. The importance of the latter is that it could provide an in vitro model for cellular immunity directed specifically towards the epidermis in vivo. This might include intraepidermal autoimmune dermatites like some forms of pemphigus, reactions to tumor specific antigens in skin carcinoma or premalignant epidermal lesions, and the tissue specific component of skin allograft rejection, i.e. reactions to the "Sk" or skin specific histocompatibility alloantigen (Boyse et al., Nature 227:901, 1970). The rationale for the development of in vitro models for these reactions is the general one for the development of any in vitro model; namely, the establishment of a system in which factors presumed to influence reactions in vivo can be controlled and investigated systematically. In addition, a particular advantage of a rapid, in vitro assay utilizing freshly explanted epidermal cells as targets is that it allows for a closer approximation of the susceptibility of these cells to immune damage in situ than long-term assays or those using long-term cultured cells as targets. This work supports Objective 5(B), Approaches 4 and 2.

Proposed Course of Project: Having demonstrated the feasibility of the model, attention will now be focused on using it to study reactions to the Sk histocompatibility antigen mentioned above. Mouse radiation chimeras will be produced which are immunologically tolerant to allogeneic lymphoid cells, but which vigorously reject skin allografts from the same donor strain. The cytotoxic response of the hosts' lymphoid cells to donor epidermal and lymphoid cells will then be assayed in vitro. In addition, possibility of using the model to assay the response to tumor specific antigens in chemically-induced mouse skin carcinoma will be explored.

Honors and Awards: None

Publications: None

Key Words: Epidermal cells; Cell-mediated immunity; Trypsinization; Chromium-release; Microtiter trays; Allogeneic; Tumor antigens

Serial No. NCI Z01 CBD 5045(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic Control of Cell Mediated Immunity to NIP-Modified Syngeneic Lymphocyte Proteins

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: Terry G. Rehn, M.D.; Hillel Koren, Ph.D.

Cooperating Units: John Inman, Ph.D., Laboratory of Immunology, NIAID, NIH

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: The purpose of this project was to investigate *in vitro* induction of cell mediated immunity, i.e., cell mediated lympholysis (CML) and mixed lymphocyte reactivity (MLR) of murine lymphocytes to syngeneic and allogeneic lymphoid cells (normal and neoplastic) modified with trinitrophenol (TNPO). This study was designed to establish: a) whether the immune reactivity obtained was specific for the haptenic moiety (TNP) or whether it involved reactivity to some TNP-modified cell surface protein(s); b) whether response potential in this model was associated with an immune response (Ir) gene, and if so to determine the linkage pattern; c) how specific the reactivity is *vis a vis* the genetic and cellular components of the induction and effector phases; d) if the immune reaction is composed exclusively of T cells or if B cells are also required; and e) whether antibodies specific for TNP are generated in addition to the cell mediated response during the course of immunization.

Methods Employed: Similar to those outlined in NCI-5038 except the NIP hapten was used instead of TNP.

Major Findings: 1) Cell mediated cytotoxicity to NIP-modified syngeneic spleen cells was obtained which was specific for NIP-modified K and D H-2 serological region gene products on lymphocyte surfaces. 2) Weak but significant mixed lymphocyte reactivity was obtained to NIP-modified syngeneic lymphocytes. 3) H-2 linked Ir genes appear to control cell mediated immunity to the new antigenic determinants.

Significance for Cancer Research and Program of the Institute: This project is of basic immunological interest in that it describes a new murine major histocompatibility-linked immunological phenomenon. The fact that the phenomenon itself involves immunological reactivity to modified self lymphocyte surface proteins raises the possibility that this is a type of autoimmunity which is H-2 linked. This observation could provide an initial model for investigating the immunological effects of autoimmune and viral-modified lymphocyte surface proteins. This model represents the first demonstration of major histocompatibility control of the formation of new cell bound modified self antigens and of the response potential to these new antigens. It has the potential to provide a working model for bifunctional major histocompatibility control of disease susceptibility in those examples of HL-A-associated disease. This work supports Objective 2, Approach 5.

Proposed Course of Project: Similar to NCI Z01 CBD 5038(c)-02 I.

Honors and Awards: None

Publications: None

Key Words: Genetic Control; Cell Mediated Immunity; Modified Syngeneic Lymphocyte Proteins; Cell mediated lympholysis; Mixed lymphocyte reactivity; Syngeneic; Allogeneic; Lymphoid cells; Normal; Neoplastic; Immune response (Ir) gene; T cells.

Serial No. NCI Z01 CBD 5046(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic Control of In Vivo Cell Mediated Immunity to Synthetic Polypeptides and Natural Proteins in Mice.

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: David Sachs, M.D. and Anne-Marie Schmitt, Ph.D.

Cooperating Units: Stephen David, M.D., Viral Biology Branch, NCI; Edna Mozes, Ph.D., Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel; Michael Sela, Ph.D., Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Man Years:

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Objectives: H-2 linked genetic control of antibody responsiveness has been demonstrated for a number of synthetic and natural immunogens, including the synthetic polypeptide (T,G)-A--L and staphylococcal nuclease (for review, see Advanc. Immunol. 11: 31, 1969). The purpose of this study was to determine whether a) similar genetic control of cell mediated immunity in vivo could be demonstrated for (T,G)-A--L and nuclease; b) such cell mediated immunity is generated by T- or B-lymphocytes; and c) cell mediated immunity can be demonstrated for T-cell independent synthetic polypeptide immunogens which are under genetic control.

Methods Employed: A footpad assay involving vascular leakage at a site of inflammation has been developed to study murine cell mediated immunity to the synthetic polypeptide GAT (Davis, Scand. J. Immunol., in press). In the current study mice injected with the above-mentioned immunogens were challenged in one hind footpad with the immunogen, followed by an intraperitoneal injection of ^{125}I-labelled serum albumin. One day later the mice were killed, and the relative amounts of isotope leakage in the control and experimental feet were compared. Adoptive transfers of T- and B-lymphocytes from immunized mice were also made in untreated hosts.

Major Findings: Cell mediated immunity as assessed by the footpad assay was obtained to (T,G)-A--L and nuclease. The phenomenon was controlled by H-2 linked Ir genes and the strain distribution for both immunogens were identical to that seen for antibody production. The phenomenon was transferrable by T-lymphocyte but not by B-cells from immunized animals.

Significance for Cancer Research and the Program of the Institute: These observations indicate that T-cell mediated immunity in the mouse is controlled by H-2 linked immune response genes. Such genes may have relevance for the individual's susceptibility or resistance to diseases. This work supports Objective 2, Approach 5.

Proposed Course of Project: Further genetic mapping studies will be required using genetic recombinant strains to determine if the genes controlling cell mediated immunity map in the same region as those controlling antibody synthesis. Additional specificity studies will be performed using: a) synthetic polypeptides which partially cross-react with (T,G)-A--L; and b) peptide fragments of nuclease.

Honors and Awards: None

Publications: None

Key Words: Genetic Control; Cell Mediated Immunity; H-2 linked genetic control; Genetic control; T- or B-lymphocytes.

Serial No. NCI Z01 CBD 5047(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Serological and Chemical Analysis of Ia Antigens: Implications for Genetic Fine Structure and Function of the Immune Response (I) Region of the Mouse MHC

Previous Serial Number: None

Principal Investigator: Susan Cullen, Ph.D.

Other Investigators: David H. Sachs, M.D., Chella S. David, Ph.D.

Cooperating Units: Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor, Michigan

Man Years:

Total:	7/10
Professional:	7/10
Other:	0

Project Description:

Objectives: The purpose of this project is to gain a better understanding of the genetic fine structure of the immune response (I) region of the mouse major histocompatibility complex (MHC) and to investigate some of its gene products.

Besides controlling several types of immune functions through the Ir genes, MLR genes and GVH genes, the I region determines the presence of a series of alloantigenic specificities on the surfaces of lymphoid cells. Recombination has separated some of these determinants, thus enabling a subdivision of the I region into three subregions to each of which several of the known Ia specificities can be assigned. Several problems exist which are the subject of the present project. (1) Solubilized Ia antigens which map in different subregions have been shown to be separable by indirect immune precipitation (Cullen et al., Proc. Nat. Acad. Sci. 71:648, 1974; Cullen and Nathenson in The Immune Response: Genes, Receptors, Signals, ed. Sercarz and Williamson, Academic Press, 1974, p. 191; Sachs et al., Transplantation, in press). It is of interest to find out whether antigenic specificities mapping in the same subregion would also be separable or would be coprecipitable. (2) Newly described Ia antigenic specificities require examination to determine whether they are chemically similar to previously described specificities. In addition, the precipitation technique may be useful to predict the subregion in which the new specificities may map. Recombinants appropriate for mapping of the new specificities are so far unavailable. (3) The antigens should be further characterized chemically. (4) As a long-range goal, possible association between the Ia specificities and molecules mediating the I region immune functions could be investigated.

Methods Employed: Radiolabelled detergent solubilized antigen preparations made from mouse spleen cells are the materials analyzed. Antisera have been raised by Dr. David and Dr. Sachs. The specific antigen molecules are isolated by indirect immune precipitation and the components precipitated are analyzed by SDS-polyacrylamide gel electrophoresis. Other separation methods employing gel filtration or charge dependent separation (ion exchange chromatography, isoelectric focusing may be employed. Affinity chromatography with plant lectins is being used to isolate glycoproteins.

Major Findings: 1) In strains B10.D2 and B10, specificities mapping in the I-A subregion were examined. In strain B10.D2, Ia specificities 8 and 11 both map in the same region. These specificities are coprecipitable, i.e., anti-8 antiserum precipitates specificities 11 and 8. In strain B10, it has been shown that specificities 8 and 9 are coprecipitable. 2) An attempt was made to test two specificities (5 and 7) mapping in the I-C region of P/J strain mice. These specificities precipitated independently. Further genetic studies by Dr. David on a hitherto unexamined recombinant have indicated reassociation of 5 + 7 and therefore that these two specificities may actually be in different subregions. 3) The strain P/J has a new Ia specificity (Ia.13) which is as yet unmapped. This specificity coprecipitates with Ia.7, indicating that it may map in the same region as does Ia.7 (I-C). 4) The Ia antigens isolated by indirect immune precipitation show a complex pattern on SDS-polyacrylamide gel electrophoresis. Two or three bands in the molecular weight range 28,000-35,000 are found.

Significance for Cancer Research and the Program of the Institute: The study of the organization of the I genetic region and of the structure of its gene products has fundamental importance for a greater understanding of the control of the immune response. The immunologic mechanisms involved affect several areas of biomedical research including transplantation, immune deficiency disease, autoimmune disease, and, of course, immunological approaches to cancer treatment. A direct result of this study should be more detailed knowledge of the complexity of the I region, and this should enable finer distinctions to be made in correlating structures with function. If the Ia antigens themselves are involved in the control of the immune response, greater knowledge of their chemical nature will be greatly needed. This work supports Objective 2, Approach 1.

Proposed Course of Project: The precipitation behavior of Ia specificities in F_1 hybrids will be examined. Several new Ia specificities will be analyzed with attempts to predict their map position by coprecipitation methods. The heterogeneous gel electrophoresis patterns found for Ia antigens will be analyzed to try to determine whether the multiple peaks observed reflect true molecular complexity.

Honors and Awards: None

Publications: None

Key Words: Immune response genes, immune response associated antigens, major histocompatibility complex.

Serial No. NCI Z01 CBD 5048(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Attempted Isolation of Fc Receptor: Structural Relationship to Ia Antigen Molecules

Previous Serial Number: None

Principal Investigators: Susan Cullen, Ph.D., Howard Dickler, M.D.

Other Investigators: David H. Sachs, M.D., Pierre Henkart, Ph.D.

Cooperating Units: None

Man Years:

Total	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives: Anti-Ia antisera have been shown capable of blocking binding of fluoresceinated Ig aggregates to lymphocytes. This blocking of Fc receptor activity was specific for anti-Ia antibody and was not due to anti-H-2 antibody (Dickler and Sachs, J. Exp. Med. 140:779, 1974). In view of these findings, it was deemed worthwhile to attempt isolation of Fc receptors to determine whether they were structurally associated with Ia antigen molecules or merely fortuitously close on the cell surface.

Methods Employed: Binding to immobilized Ig aggregates was the method chosen to isolate Fc receptor. Spleen cells or cell lines were radiolabelled with ^{3}H-leucine, and the labelled cells were detergent solubilized. The detergent-soluble material was then used as a source of labelled Fc receptor.

Major Findings: At first it appeared that the binding of labelled material by Ig-aggregate-Sepharose was comparable to binding by the adsorption control, BSA-aggregate-Sepharose. The material eluted from the Sepharose beads by boiling in SDS and 2-mercaptoethanol appeared similar for both Ig and BSA aggregate adsorptions. However, one experiment showed that if the labelled preparation was incubated with several batches of BSA aggregate-Sepharose, the level of "nonspecific" sticking was sufficiently reduced to allow observation of some material adsorbing to the Ig aggregate-Sepharose but not to BSA aggregate Sepharose.

On SDS disc gel electrophoresis, this Ig binding material appeared as three peaks of ∿70,000, ∿22,000 and ∿20,000 MW.

Significance for Cancer Research and the Program of the Institute: It is important to know whether the association noted between Ia and Fc receptor when tested at the cell surface is a reflection of some physical bond between these entities at the molecular level. This distinction is of interest to help uncover the means by which the I genetic region which determines Ia antigens also has a major role in several immune response functions. This work supports Objective 2, Approach 1.

Proposed Course of Project: The preliminary result mentioned above requires much repetition and improvement. Further attempts will be made to eliminate nonspecifically sticky material. A control of $F(ab)_2$ aggregate-Sepharose will be introduced. Other possible artefacts such as sticking of labelled IgM will be examined.

Honors and Awards: None

Publications: None

Key Words: Fc receptor, immune response genes, immune response associated antigens

Serial No. NCI Z01 CBD 5049(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: *In Vitro* Induction of Cell Mediated Cytotoxicity of F_1 Lymphocytes Against Parental Cells Expressing Hemopoietic Histocompatibility Antigens

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: None

Cooperating Units: Gustavo Cudkowicz, M.D., Department of Pathology, State University of New York, Buffalo, New York

Man Years:

Total:	1/8
Professional:	1/16
Other:	1/16

Project Description:

Objectives: Resistance of irradiated mice to proliferation of parental or allogeneic hemopoietic cells is regulated by multiple H-2 linked genes (Cudkowicz, In Genetic Control of Immune Responsiveness, McDevitt and Landy, Academic Press, 1972). These hemopoietic histocompatibility (Hh) genes are phenotypically expressed only in blood-forming and leukemic cells, are not codominantly inherited, and their products are capable of stimulating host-vs-graft reactions which are radioresistant *in vivo*. The objective of this study was to determine if cell mediated cytotoxicity (CMC) could be generated *in vitro* in F_1 lymphocytes against parental cells; if so, to establish if the response involves reactivity against Hh determinants.

Methods Employed: (B10xB10.A)F_1 spleen cells were sensitized in culture against B10 and B10.A parental spleen cells. The effector cells were assayed on ^{51}Cr-labelled tumor target cells expressing H-2^a and H-2^b alleles.

Major Findings: Similar to the *in vivo* F_1 hemopoietic graft rejection of parental cells, F_1 responding lymphocytes generated cytotoxic effector cells against spleen cells of the H-2^b parent but not against those of the H-2^a parent.

Significance for Cancer Research and the Program of the Institute: Since the genes are expressed on both normal and neoplastic hemopoietic cells, this study may indicate some of the genetic and antigenic parameters involved in surveillance against leukemias. The adaptation of an *in vitro* model for such studies permits analysis and dissection of these parameters not heretofore possible *in vivo*. The development of the *in vitro* system permits an investigation of possible Hh genes in humans. This work supports Objective 6 and 2, Approach 4 and 5.

Proposed Course of Project: The *in vitro* cytotoxic model will be used to investigate a number of other F_1 and parental combinations and compare them with the known *in vivo* studies. Leukemic and normal marrow cells will be investigated as possible stimulator and target cells. Other established parameters of *in vivo* hybrid resistance will be compared with the *in vitro* system. Effector cell generated *in vitro* will be used *in vivo* to determine if they have any protective effect against leukemias.

Honors and Awards: None

Publications: None

Key Words: Cell Mediated Cytotoxicity; Hemopoietic Histocompatibility Antigens; Resistance of; Hemopoietic cells; Hemopoietic histocompatbility (Hh) genes; Blood-forming and leukemic cells; Cell mediated cytotoxicity; F_1 lymphocytes against parental cells.

Serial No. NCI Z01 CBD 5050(c)-01 I
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Constant Portions of Immunoglobulins

Previous Serial Number: None

Principal Investigator: David M. Segal, Ph.D.

Other Investigators: None

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives: To develop assays for the interactions of the constant domains of immunoglobulins with the various effector systems employed in the immune reactions. To study the relationship of antigenic recognition to these interactions and to study the molecular basis of these phenomena.

Methods Employed: Organic synthesis, gel filtration, ion exchange chromatography, affinity labelling, affinity chromatography, polyacrylamide gel electrophoresis, complement fixation.

Major Findings: None. Project still in initial stages.

Significance for Cancer Research and Program of the Institute: The recognition of foreign substances (antigens) by immunoglobulins elicits a number of reactions which normally lead to their elimination from the body. Previous work by the principal investigator has contributed to knowledge of the molecular basis of antigenic recognition. This project proposes to investigate the ways in which recognition elicits the protective responses of immunity. It is hoped that these studies will enhance our understanding of these processes and allow us to better control the immune reaction. This work supports Objective 6, Approaches 3 and 4.

Proposed Course of Project: An IgM myeloma protein with anti-DNP activity will be labelled by a series of bivalent affinity labels. The interaction of the labelled protein with the first component of complement (C1), and with lymphocytes if possible, will be studied.

Attempts will also be made to isolate C1 in the zymagen form, in order to use it as an assay for "turned on" immunoglobulin.

It is also expected that these studies will be performed using the other classes of immunoglobulin.

Honors and Awards: None

Publications: None

Key Words: constant domains; immunoglobulins; antigenic recognition; complement fixation.

LABORATORY OF IMMUNODIAGNOSIS

SUMMARY

July 1, 1974 - June 30, 1975

The Laboratory of Immunodiagnosis was initiated in December, 1974. This was effected mainly by transfer of staff from the Cellular and Tumor Immunology Section, Laboratory of Cell Biology. During this past year, studies on analysis of cell-mediated immunity to tumor associated antigens have continued. In addition, an increasing emphasis is being placed on the immunodiagnostic implications of this work and on the development of new procedures for the immunodiagnosis of cancer and for reliable monitoring of disease in tumor-bearing individuals.

Some highlights of achievements in the past year:

I. Tumor immunity in animal tumor systems.

Extensive studies have been performed with C57BL/6 mice inoculated with murine sarcoma virus (MSV). Most stocks of MSV cause temporary tumor growth in adult mice and then regression. Stocks of MSV have also been found which reliably produce progressive tumor growth. It has therefore been possible to compare the patterns of immunity in regressors and progressors. The kinetics of the antibody response to both virus preparations were biphasic; the level of the antibody first peaked at day 10, declined and became undetectable around day 14, then rose steadily after day 20. In the regressors, the antibody level started to decline after 60 days, but in the progressors, the levels remained high. With regressor stocks of virus, high cell-mediated cytotoxicity could be detected, in a 4 hour ^{51}Cr release assay, at about 14 days. Reactivity then rapidly declined to low levels after 20 days. In progressors, the same kinetics of response were seen but the levels of cytotoxicity were significantly lower.

A variety of assays of cell-mediated cytotoxicity is available for study. Efforts have been made to understand the inter-relationships among these assays, and to determine the assays most useful for monitoring of cell-mediated immunity. In the MSV tumor system, we therefore undertook a direct comparison between the ^{51}Cr release assay, an ^{125}IUdR release cytotoxicity assay, and the visual microcytotoxicity assay. Essentially the same kinetics of response were seen in all three assays. A similar study has been performed in a rat tumor model. Using a chemically induced breast adenocarcinoma tumor system in Fisher rats, there does appear to be substantial differences in the cellular cytotoxicity results obtained using each of three different techniques. The ^{51}Chromium assay appears to measure early membrane damage by cytotoxic lymphocytes, whereas a longer incubation time (24-48 hrs) is required for release of the other two isotopes. The results of assays of cellular cytotoxicity obtained with I^{125} IUdR and with tritiated proline are somewhat similar though not exactly the same. Since the former isotope stops cell division in the target cell, it may allow for the expression of quantitatively different amounts of antigen on the membrane surface as compared with the tritiated proline assay.

In a Friend virus induced tumor system (FBL-3) in mice, the ^{125}IUdR assay has proven superior to the ^{51}Cr release assay. The kinetics of the primary cell-mediated cytotoxic response was biphasic: the reactivity first peaked at days 10-14, declined and became undetectable at days 20-30, and then the reactivity reappeared and persisted up to at least 60 days. The cytotoxic reactivity of first both peak (days 10-14) and second peak (days 40-60)was found to be T-cell dependent.

It has been possible to analyze the nature of the effector cells in these immune assays. In the MSV tumor system, the ^{51}Cr release assay was shown to be entirely dependent on T cells, with reactivity eliminated by treatment with anti-Θ plus complement. Removal of macrophages had no effect. A paradoxical finding has been that a majority of the cytotoxic cells were consistently retained on Sephadex-anti-gamma globulin columns and recovered in the "B cell" function. It seems likely that the cytotoxic cells are T cells with some immunoglobulin on their surface. These findings are consistent with other observations that activated T cells may have surface receptors for immunoglobulins. In the FBL-3 tumor system, T cells were also responsible for the cytotoxicity induced by tumor cell inoculation.

Extensive studies have been performed on the cryopreservation of effector cells and of target cells in these systems. This has proven extremely useful in standardizing the assay and minimizing day-to-day variations. This has also allowed us to rapidly detect changes in the antigenicity and susceptibility to lysis of fresh target cells.

An apparent paradox in the MSV tumor system and other syngeneic systems has been the short-lived cytotoxic cellular immune response, in contrast to the long lasting in vivo resistance to tumor challenge. We have recently demonstrated that challenge with tumor cells carrying the appropriate antigens, even many months after primary inoculation with MSV, resulted in rapid appearance of a secondary cytotoxic response. High levels of cytotoxic reactivity could be consistently detected, mainly in the region of tumor cell challenge. Extensive studies of the secondary response to FBL-3 have also been performed. It was found that the secondary response peaked earlier (days 3-5), the reactivity was higher and persisted longer than the primary response. The secondary cytotoxic response in both the MSV and FBL-3 systems was found to be T cell dependent.

Inoculation of immune lymphoid cells with tumor cells also resulted in the generation of an in vitro secondary cytotoxic response in both of these tumor systems. Strong cytotoxicity could be demonstrated, even at low effector:target cell ratios.

Re-challenge of previously inoculated rats with Gross virus-induced tumor cells also resulted in the rapid appearance of cytotoxic reactivity.

In contrast to the mouse, this secondary response was more generalized, with strong activity in the spleen as well as in the region of tumor challenge. *In vitro* incubation of immune cells with tumor cells also resulted in a brisk secondary cytotoxic response.

A major concern in these studies has been the specificity of the reactions, and the relationship of the antigens detected to transplantation antigens which can induce resistance to tumor growth. *In vivo* tumor transplantation experiments showed that only the F and FMR antigens could function as transplantation antigens. Testing by the ^{125}IUdR release assay, the specificity of the primary cytotoxic response was also mostly against F and FMR antigen, with very little reactivity against non-FMR antigens. When the specificity of the secondary response was studied, the same observations were obtained and this gave very good correlation with the *in vivo* tumor immunity. A competitive inhibition assay has been developed to analyze the specificity of the ^{51}Cr release cytotoxicity assay. Studies of the MSV system have indicated that the antigens detected were not directly related to MSV or FMR leukemia antigens, but rather to mouse endogenous type C viruses. Many mouse tumor cell lines *in vivo*, and particularly *in vitro*, induced by a variety of methods or agents, have been shown to contain this antigen, designated MEV-SA1, and other markers of endogenous viruses. Infectious endogenous viruses could be isolated from most of these cells and these could infect mouse embryo fibroblasts or xenogeneic cultured cells. Upon infection of these cells with the endogenous viruses, expression of MEV-SA1 could be detected, confirming the direct association of this antigen with mouse endogenous type C viruses. The endogenous virus associated antigens have been found to also act as transplantation antigens in the MSV system.

Cell-mediated immunity could also be detected in this system by the lymphocyte stimulation assay. Low levels of stimulation were seen when lymphocytes from mice inoculated 14 days before with MSV were cultured with mitomycin treated Rauscher leukemia cells. Suppressor cells in the spleens of these tumor bearing mice were shown to partially inhibit this stimulation by tumor cells, inhibited the *in vitro* generation of cytoxic cells, and also depressed reactivity to mitogens and to allogeneic cells. Extensive studies have been performed to characterize the mechanism of this suppression and the nature of the cells mediating these effects. Considerable evidence has indicated that macrophages are responsible for the suppression. These suppressor macrophages were found to have a variety of effects. In addition to depressing proliferation of lymphocytes, they have also been shown to have potent inhibitory effects on the *in vitro* growth of tumor cells. These growth inhibitory effects were nonspecific, in that a wide variety of tumor cells were equally affected. Potent suppressor cells could be induced not only by MSV tumor growth, but also by growth of a variety of other mouse tumors and by inoculation of *Corynebacterium parvum*.

In addition to suppressor macrophages, it was found that some tumor cells themselves could inhibit lymphocyte stimulation reactions. This tumor cell-mediated depression has been found to be mediated by cell-free filtrates derived from the tumor cells. Current evidence suggests that it is due to an infectious agent, presumably a virus; inhibitory activity is sedimentable at 100,000g, is inactivated by ultraviolet irradiation, and can be neutralized by ascites fluid from mice carrying the tumors. Both the suppressor cells and the inhibitory factors within tumors may account for some of the immunodepression associated with tumors, and may also explain many of the negative cellular immunity reactions seen in tumor systems. In a Gross virus-induced tumor system in rats with progressive tumor growth, no positive lymphocyte stimulation was seen. These tumor bearing rats were also found to have depressed responses to mitogens. As with the MSV system in mice, this lack of stimulation in progressor rats was found to be due to suppressor cells. After removal of suppressor cells on adherence columns, positive stimulation by tumor cells could be detected. In addition to the suppressor cells, high concentrations of (C58NT)D tumor cells themselves were shown to inhibit lymphocyte stimulation. This effect seems to be the same as that described above for mouse tumor cells. Both types of inhibitory phenomena have also been found in the rat mammary tumor system.

Lymphoid cells from many normal rats and mice, of a variety of inbred and random bred strains, were found to have reactivity, in the ^{51}Cr release cytotoxicity assay, against several syngeneic and allogeneic tumors. In both rats and mice, peak reactivity was seen in young animals, usually between 5-8 weeks of age. Older animals had little or no demonstrable reactivity. In the rats, this activity could be shown to be independent of T cells and macrophages. The characteristics of the reactivity in the mouse have been studied in depth. Very high reactivity was seen with cells from athymic, nude mice, suggesting that the mouse's natural reactivity was also independent of T cells. In addition, treatment of cells with anti-O plus complement had little effect, and removal of macrophages also did not inhibit reactivity. No cell surface markers could be found on the natural cytotoxic cells, and it has been concluded they represent a novel subpopulation of lymphoid cells, tentatively designated N cells. Target cells susceptible to lysis included tumors induced by oncogenic type C viruses but also tumors induced by other means and expressing endogenous type C viruses. By use of the inhibition assay, the natural cytotoxic reactivity in both mice and rats could be shown to be related to expression of endogenous type C viruses, but the specificities were distinct from those recognized by cells from deliberately immunized animals.

Of central importance in studies of tumor immunity is the relationship of the results obtained in in vitro assays to the in vivo response to tumor growth. An adoptive transfer system has, therefore, been established to determine the effect of various cell populations, at various times after immunization, in resistance against tumor growth. Cells from regressor mice, from 14-40 days after MSV inoculation, protected recipients completely from MSV tumor induction. Cells at 14 days after MSV also protected very well against challenge with a Rauscher virus-induced tumor. Cells from progressor mice at 14 days also gave some protection against MSV and tumor cells. It should be noted that these cells were taken at the peak of the in vitro cytotoxic response. The in vivo effects of suppressor cells are also currently being investigated in this system.

Adoptive transfer experiments have also been prepared in the FBL-3 tumor system. Although lymphocytes from progressor mice had some cytotoxic reactivity, it was 3-10 times lower than that of cells from regressor mice. When these lymphocytes were used in adoptive transfer experiments, the regressors' lymphocytes gave 90% protection against tumor (FBL-3) challenge, whereas the progressors' lymphocytes only gave 40% protection.

II. Tumor immunity in man.

A series of assays for measuring the general cell-mediated immunocompetence of cancer patients have been established. These assays are: enumeration of the percent of T cells in peripheral blood by rosette formation with sheep erythrocytes, cytotoxic reactivity against human lymphoid cell lines, and lymphocyte stimulation by mitogens, antigens, and mixed lymphocyte cultures. Continued use of cryopreserved cells in these assays have allowed us to standardize the day-to-day variation to a large degree. Assays for rosette formation with sheep erythrocytes, performed under suboptimal conditions, at 29°C, were found to be particularly discriminatory between cells from cancer patients and cells from controls. A large proportion of patients with carcinomas, sarcomas, and melanomas were found to have significantly depressed levels of rosette forming cells (RFC) in this assay. Both patients with localized disease (77%) and those with metastatic disease (100%) had levels of RFC significantly below those of normal controls. In contrast, only 10% of normal individuals had significantly depressed percentages of RFC. This assay is still under investigation regarding its potential application to the immunodiagnosis of cancer, and the monitoring of the clinical course of disease in cancer patients. Studies are also being performed to determine the correlation between % RFC and functional activity in assays of cell-mediated immunity.

An additional, intriguing observation made with the rosette assays has been that malignant pleural effusions contained high percentages of RFC, in the face of depressed levels in the peripheral blood. The significance of this consistent observation is actively being investigated.

The ability of human T cells to form rosettes with sheep erthrocytes has also led to a very useful method for isolation of T cells from peripheral blood leukocytes. Fractions with high yield and purity have been obtained. In assays of lymphocyte stimulation, reactivity to the mitogens PHA and concanavalin A, and to the antigens PPD and tetanus, and to allogeneic leukocytes has been completely restricted to the T cell fraction. Different results were seen with a staphylococcal antigen; this produced strong stimulation of non-T cells as well as T cells. It seems likely that this antigen represents one of the first strong stimulants for human B cells or other non-T cells. In assays of cytotoxicity against human lymphoid cell lines, both T and non-T cells were also found to be reactive.

In addition, to the rosette assay, cancer patients, especially those with larger tumor burdens, tend to show poor response to mitogens and to alloantigens in the mixed lymphocyte culture. These assays are currently being used to monitor patients on immunotherapy and to determine if they are helpful in the clinical evaluation of such patients.

Further studies have been peformed with the direct leukocyte migration inhibition assay. The leukocytes of patients with carcinoma of the breast, malignant melanoma, carcinoma of the lung, and Ewing's sarcoma have been shown to be specifically inhibited by 3M KCl extracts of autologous and allogeneic tumors of the same histological type. Little cross-reactivity of patients with other types of cancer or of normal controls has been seen.

It has recently been reported that some patients with breast cancer can have specific cell-mediated immunity to antigens associated with mouse mammary tumor virus (MTV). We have performed a series of experiments to evaluate this. We have, indeed, found that a high proportion of breast cancer patients, and not normal individuals or other cancer patients, react with MTV and not with control mouse materials. Studies are being actively pursued to determine the significance of this observation.

In earlier studies, we found that allogeneic extracts of tumors were not useful for study of stimulation by tumor associated antigens, since normal alloantigens could also be recognized in these extracts. In the past year, a major effort has been made to study stimulation of cancer patients' lymphocytes by autologous tumor cells and by membrane extracts and 3M KCl extracts of autologous tumors. In approximately 50% of the patients tested thus far, stimulation has been seen with tumor materials and not with normal control tissues. The membrane extracts appeared particularly promising, in that very low concentrations gave positive results.

Patients with cancer have been shown to have delayed hypersensitivity reactions to extracts of tumor cells and of some tissue culture cell lines derived from tumors. A prospective study of reactivity of African patients with Burkitt's tumors is being performed with extracts from a cell line derived from a Burkitt tumor. This study has now been in progress for over a year and the results are quite interesting. Reactivity to this extract has correlated well with clinical status. Before therapy, only patients with small localized tumors have reacted. When patients have been successfully treated and then tested in remission, a high proportion have been reactive. Patients failing to go into remission, or patients with early relapse have tended to be unreactive, although they were not anergic. The skin reactivity has been correlated with serological studies of antibodies to EBV associated antigens, and a very significant inverse correlation between skin reactivity to the cell line extract and antibodies to EBV nuclear antigen has been found.

Efforts have been made to solubilize and isolate the skin reactive antigens from some tumors. In malignant melanoma, it has been possible to prepare semi-purified antigens which give a high proportion of reactions in melanoma patients and little reactivity in patients with other types of cancer. The possible diagnostic potential of this antigen is being evaluated in patients suspected of having ocular melanoma. Thus far, skin tests with this antigen have been able to accurately distinguish patients with ocular melanoma from those with benign, simulating lesions of the eye or with metastatic cancer in the eye. The separated melanoma antigens have also been found to give positive reactions in the leukocyte migration assay, so that it may be possible to perform more extensive tests *in vitro* to evaluate the diagnostic potential of this reactivity.

Project No. Z01 CBD 08500-01 LID
1. Laboratory of Immunodiagnosis
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell-mediated immune response in experimental animal systems and immunological monitoring in human tumor systems.

Previous Serial Number: None

Principal Investigator: Dr. Robert K. Oldham

Other Investigators:
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Cooperating Units: Surgery Branch, NCI; Radiation Therapy Branch, NCI; Viral Leukemia and Lymphoma Branch, NCI; Viral Biology Branch, NCI; Epidemiology Branch, NCI; Pediatric Oncology Branch, NCI; George Washington University; Georgetown University School of Medicine; Hematology-Oncology Sections at the National Naval Medical Center; Walter Reed Army Medical Center; Portsmouth Naval Hospital; University of South Carolina; and Litton-Bionetics, Inc.

Man Years:
Total - 4.25
Professional - 3.25
Other - 1.00

Project Description:

Objectives: The objectives of this project are: (1) to detect tumor specific and tumor associated antigens and to quantitate these as well as normal antigens on tumor cells; (2) to detect cellular immune reactions to tumor antigens; (3) to evaluate the diagnostic, prognostic and therapeutic significance of cellular immune responses to tumor antigens; (4) to develop and evaluate assays for the immunological monitoring and immunodiagnosis of human cancer; (5) to isolate and characterize tumor antigens and other cell surface antigens; (6) to test the reagents developed under (5) in certain *in vitro* assays as well as in delayed hypersensitivity reactions in these tumor systems; (7) to monitor general immunological competence in cancer bearers; (8) to test the therapeutic efficacy of certain immunological stimulants and tumor associated antigens in animal and human tumor systems relative to the clinical state of the host as well as relative to the effect on these assays mentioned above; (9) to evaluate the effects on the immune response of inhibitory phenomena associated with tumor cells and tumor cell growth.

Methods Employed: Lymphocytes and tumor cells are obtained from patients and controls. Normal and tumor cell lines are maintained in tissue culture. Lymphocytes and tumor cells are stored in a viable frozen state. Inbred strains of rats and transplantable tumors induced by viral, chemical or unknown agents are used as model systems. Cellular immune reactions are measured by lymphocyte-mediated cellular cytotoxicity assays (visual, ^{51}Chromium, I^{125} IUdR, ^{3}H-proline assays), migration inhibition, mixed lymphocyte cultures, and skin tests. Human and animal tumors as well as normal cell membrane and soluble fractions are prepared for antigenic and biochemical studies. Rosettes are tested for by sheep erythrocyte techniques. Inhibitory phenomena are assayed by the inhibition of mitogen stimulanted lymphocyte transformation.

Major Findings:

I. Tumor Immunity in Animal Models:

a. Comparison of Assays of Cellular Cytotoxicity.

Multiple laboratories use different assays of cell-mediated cytotoxicity. We have attempted to determine the relationship between several of these assays (^{51}Chromium, I^{125} IUdR, and ^{3}H-proline) in a rat tumor model carried in our laboratory. Using a chemically induced breast adenocarcinoma tumor system in Fisher rats, as well as a Gross virus-induced lymphoma, there does appear to be substantial differences in the cellular cytotoxicity results obtained using each of three techniques. The ^{51}Chromium assay appears to measure early membrane damage by cytotoxic lymphocytes whereas a longer incubation time (24-48 hrs) is required for release of the other two isotopes. The results of assays of cellular cytotoxicity obtained with I^{125} IUdR and with tritiated proline are somewhat similar though not exactly the same. Since the former isotope stops cell division in the target cell, it may allow for the expression of quantitatively different amounts of antigen on the membrane surface as compared with the tritiated proline assay. This may account for the quantitative differences observed between the two assays. There are in addition to the above, multiple technical differences between the assays which are of interest to the investigators using such assays.

b. Evaluation of Normal Activity.

In our rat tumor models, the activity of unimmunized rats in comparison to immunized rats has been assayed utilizing each of the three cell-mediated cytotoxicity assays detailed under "a". It is apparent that unimmunized animals have appreciable normal activity against several rat tumor cell lines. This normal activity can be assayed for by any of the three assays. This normal activity has a definite relationship to the age of the animal as well as having some individual variability from animal to animal at the same age. The continuing characterization of this normal activity is of tremendous importance in evaluating cellular cytotoxicity in all animal tumor systems as well as in the human.

c. In Vitro Versus In Vivo Carried Tumor Cells - Their Use in Immunological Assays.

Comparison of the ascites passage of the rat Gross virus-induced lymphoma, (C58NT)D, with the same cell line carried in tissue culture revealed both to act as satisfactory immunogens and targets for cellular cytotoxicity. Certain differences appeared in the tissue culture line as opposed to the ascites line. We are currently characterizing some of these changes by reinoculating the tissue culture line into rats and testing its efficacy as an immunizing cell as well as a target cell in the in vitro assays. Our results indicate that certain tumor associated antigens appear to be more strongly expressed in the ascites line than in the tissue culture line and that the tissue culture line when reinoculated in vivo can show some reexpression of this activity.

d. Inhibitory Phenomena.

Considerable effort has been expended in this laboratory in the past year to characterize certain inhibitory phenomena associated with tumor cell growth. In the rat mammary tumor system, at least two types of inhibition were observable in tumor bearing rats. One was direct inhibition by tumor cells or extracts of tumor cells on lymphocyte transformation by mitogens. The other was the inhibition by macrophages in the absence of tumor cells from the spleens of tumor bearing animals. The effects by splenic macrophages were lost by sonication and freeze-thawing, whereas the same treatment of the tumor cells had no effect on their inhibitory activity. Characterization of this inhibition and its relationship to the detection of cellular immunity to tumor associated antigens and to immunogenicity of the tumor cells is currently underway.

e. Cryopreservation.

The use of frozen cells is of paramount importance in the standardization of these assays. In the rat animal tumor models, we have demonstrated that cellular cytotoxicity can be cryopreserved. Active lymphocytes frozen and thawed by particular techniques can retain up to 75% of their original activity as fresh cells in cellular cytotoxicity. The use of such frozen cells will allow us to control for day-to-day variation of cellular cytotoxicity assays and will also provide standard cells for the inhibition assays described below.

f. Inhibition of Cell-Mediated Cytotoxicity.

The assay by which unlabelled target cells are added to a cell-mediated cytotoxicity assay to competitively inhibit the cytotoxicity observed has been developed in this laboratory. We have attempted in the last year to standardize this assay with the use of frozen attacker/target cell combinations and utilizing a variety of fresh and frozen cells as inhibitor cells. In this way it seems possible to define the antigens on target cells by their ability to inhibit direct cytotoxicity. The use of frozen cells should provide a reproducible level of cytotoxicity to inhibit from day-to-day. The inhibition assay has been of tremendous importance in defining the antigens involved in certain of the direct cytotoxicity assays. Whereas the initial results suggested that the cytotoxicity was due to tumor associated antigens, more recent work with the inhibition assay has suggested that endogenous type C virus in many of these cell lines is mainly responsible for antigenic specificities measured by both the direct cytotoxicity and by the inhibition assays.

g. Collaborative Research.

In addition to the above primary efforts, we are, in collaboration with the Viral Biology Branch, NCI, performing some of the assays mentioned above in immunotherapy model systems. One is the same rat chemically induced breast adenocarcinoma system described above and the other is a guinea pig chemically induced leukemia system. An associate investigator sets up animals for immunotherapy experiments and supplies our laboratory with a portion of these animals to monitor the immune response during and after immunotherapy in these animals. The purpose of the study is to see if the immunological monitoring can be related to the clinical state of the animals receiving immunotherapy. It is hoped that the immunological monitoring of immunotherapy in animal systems will shed some light on the use of various assays to monitor immunotherapy and other forms of therapy for human cancer.

II. Tumor Immunity in Man:

a. Immunocompetence of Cancer Patients.

A series of assays for measuring the general cell-mediated immunocompetence of cancer patients have been established. These assays are: 1) enumeration of the percent of T cells in peripheral blood by rosette formation with sheep erythrocytes, 2) cytotoxic reactivity against human lymphoid cell lines, and 3) lymphocyte stimulation by mitogens, antigens, and mixed lymphocyte cultures. Continued use of cryopreserved cells in these assays have allowed us to standardize the day-to-day variation in these assays to a large degree. Particularly in the rosette assay and the mixed lymphocyte culture, significant variation on a particular day of greater than 10-15% from the usual value obtained with cryopreserved cells indicated that the assay was technically inadequate on that day. The use of these assays has aided in the evaluation of the general immunological competence of human cancer patients. In general, cancer patients show a lower degree of rosette formation than normal donors. In addition, cancer patients, especially those with larger tumor burdens, tend to show lower rosette formation and poor response to mitogens and antigens in the mixed lymphocyte culture. These assays are currently being used to monitor patients on immunotherapy and to determine if they are helpful in the clinical evaluation of such patients. The cytotoxic reactivity against human lymphoid cell lines, previously reported by our laboratory to be a measure of general effector function, has been found to be influenced to a considerable extent by the stage in the cell cycle of the target cells. Therefore, some degree of standardization of the target cells is absolutely essential for using this test on a day-to-day basis to monitor general effector cell function.

b. Leukocyte Migration Inhibition Studies.

Further studies with the direct leukocyte migration inhibition assay has revealed that there is specific inhibition of migration of leukocytes from breast cancer patients and malignant melanoma patients by 3M KCl extracts of these tumors. In this assay to date, there is very little normal activity and very little cross reactivity when extracts from one tumor have been tested against the lymphocytes of patients with other tumors. These observations have been recently extended to carcinoma of the lung and to Ewing's sarcoma; there has been specific leukocyte migration inhibition with these two tumors as well.

c. Lymphocyte Stimulation by Tumor Cells and Tumor Extracts.

Lymphocyte stimulation has been performed using both whole tumor cells as well as extracts of these cells. Previously we have noted that only autologous testing is a measure of tumor associated acitivity, as allogeneic testing can also reflect stimulation by transplantation antigens. In our studies to date, approximately 50% of our tests, when an adequate number of lymphocytes were used as responding cells, demonstrated a positive autologous stimulation of patients' lymphocytes by their own tumor cells and by membrane and soluble extracts from their tumor cells. This autologous stimulation is generally at relatively low levels and has already been observed in carcinoma of the breast and carcinoma of the lung in a fairly large series of patients.

d. Assays for Cytotoxicity of Tumor Cells.

The cytotoxic reactivity of lymphocytes against tissue culture target cells has continued to be an assay plagued with certain difficulties. The specific reactivity of lymphocytes for target cells derived from tumors of similar histologic types, e.g., the reactivity of melanoma patient lymphocytes on melanoma target cells, has been seen but has not been confirmed to be an absolutely specific phenomena as defined by others. As has been noted in the animal tumor systems, considerable amounts of activity by normal controls have been noted against these cell lines and a considerable amount of cross reactivity, for example melanoma patients against a breast cancer cell line, has been noted. The presence of both normal reactivity and cross reactivity has lead us to believe that this assay is measuring the activity of lymphocytes directed against certain membrane antigens which are not specific for each tumor type, or that the individuals involved have been sensitized to a large number of antigens, including those characteristic of the tumors. Most of this cytotoxicity testing indicates that minor technical factors are not the reasons for this cross reactivity, as preparation of lymphocytes and target cells by a variety of different methods have yielded similar results. We have therefore, suggested that a major re-evaluation of cellular cytotoxicity in the human tumor systems is needed.

e. Delayed Cutaneous Hypersensitivity to Tumor Antigens.

We have continued to measure delayed hypersensitivity reactions of patients response to tumor cell extracts. We have found that the crude extracts derived by treatment of the cells with hypotonic saline solutions, as well as those crude extracts derived by KCl extractions, show much less specificity in the solid tumors (melanoma, breast, lung, and colon) than in the leukemias previously reported from this laboratory. However, an encouraging aspect of this work has been that certain of the Sephadex and polyacrylamide gel purified soluble fractions derived from these crude extracts appear to show specificity. There appears to be tumor associated activity in some fractions which can be separated from activity more generally associated with the tissue of origin. For example, in melanoma patients there is reactivity against what appears to be melanoma specific antigens as well as reactivity in different fractions against what appears to be tissue associated antigens.

f. Correlation of *In Vitro* Assays with Clinical Status of Patients.

Over the past year, we have utilized assays mentioned above in monitoring patients with neoplastic disease. This has included patients with Ewing's sarcoma, melanoma, carcinoma of the lung, carcinoma of the breast, and carcinoma of the colon. In some cases this monitoring has been done in patients receiving no specific immunotherapy and in others the monitoring has been done on patients who are being treated under an immunotherapeutic protocol in collaboration with our laboratory. Although it is too early to sharply define the relationship between the assays and the clinical state of the patient, there does appear to be some preliminary correlation between the clinical absence of tumor and the presence of activity in several of these assays of specific immune responses, as well as higher levels of general immunological competence.

III. Significance for Cancer Research.

Detection of tumor antigens and of immune reactions to tumor specific antigens is essential for progress in tumor immunology. The development and standardization of sensitive assays for cell-mediated immunity is one of the most important aspects of human tumor immunology. Because immunogenicity cannot be studied directly in the human, the evaluation of immunologic reactivity and the antigenicity of tumor cells by these assays is of paramount importance. This would fall under Objective V, Approach 4 of the National Cancer Plan.

The ability of our laboratory to perform multiple assays of cell-mediated immunity to tumor antigen should allow detailed dissection of the role of the immune response in limiting or preventing tumor growth. These assays may also be useful in the immunodiagnosis of cancer and in measuring changes of immunity in response to chemotherapy or immunotherapy. This would fall under Objective V, Approach 4 and Objective VI, Approach 1 and 4 of the National Cancer Plan.

The available immunological techniques for the solubilization and isolation of tumor associated cell surface antigens are important in their application into monitoring as described in this report. Such techniques are also perhaps useful in isolating such antigens for therapy in the future and the use of such extracts for immunotherapy would fall under Objective VI, Approach 1, 2 and 4 of the National Cancer Plan.

As described in this report, we have also been involved in immunotherapy in our laboratory. The use of various forms of immunotherapy in animal tumor models and in man, and the assessment of the efficacy of these therapeutic modalities both by clinical follow-up of the patient and by the effect on these immunological assays is of great current importance and would fall under Objective V, Approach 4 and Objective VI, Approach 1, 2 and 4 of the National Cancer Plan.

The investigation of inhibitory phenomena as it relates to the cancer cell is an area of tremendous importance. It may be the stimulation of the host immune system will be useless unless one can first rid the body of the inhibitory phenomena related to tumor cell growth. Investigations in this area would fall under Objective IV, Approach 4 and Objective VI, Approach 1, 2 and 4 of the National Cancer Plan.

IV. Proposed Course of Project.

This project represents our laboratory's continued commitment to understanding the role of the host immunological response to the tumor and tumor progression. As has been emphasized previously in this report, the interrelationship between these different assays and their relevance to the clinical state of the tumor bearer is a primary area of research for this laboratory.

In experimental models we plan to continue with immunotherapy utilizing nonspecific immunological stimulants as well as cell extracts containing tumor associated antigens in these systems. At the same time, we plan to continue our immunological monitoring in the animal tumor models and determine the relevance of this research to the human tumor system. Extraction of tumor specific antigens and characterization of the inhibitory phenomena are two specific areas in the next year that we will explore most extensively.

Our studies of the cell-mediated immunity both relative to the general immunological competence and the specific reactivity to tumor associated antigens in human tumor systems will be continued. The relationship between the assays being used in the clinical course of the patient undergoing surgery, radiation therapy, or immunotherapy will be examined. The effect of different immunotherapeutic modalities, such as BCG, Cornybacterium parvum, and tumor cell extracts will be examined through randomized clinical trials and the use of our immunological assays to monitor these patients. In addition to our collaborative work with a variety of institutions in the area, we will begin some investigation at the National Cancer Institute Clinical Center in patients with carcinoma of the lung and their ability to respond to immunotherapy and effects of such therapy on their immunological responsiveness.

Keyword Descriptors:

Tumor associated antigens-isolation, purification and specificity, immunological assays and monitoring, immunodiagnosis, immunological reactivity and immunotherapy.

Publications:

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Project No. Z01 CBD 05558-07 LID
1. Laboratory of Immunodiagnosis
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immune Response to Tumor Associated and Other Cell Surface Antigens

Previous Serial Number: NCI-5558-1

Principal Investigator: Dr. Chou-Chik Ting

Other Investigators: Dr. Ronald B. Herberman Z01 CBD 05557-07 LID
Dr. Guy Bonnard Z01 CBD 05558-07 LID

Cooperating Units: Laboratory of Cell Biology, NCI; Litton-Bionetics, Inc.; Biotech Research Laboratories, Inc.

Man Years: Total - 3.25
Professional - 2.25
Other - 1.00

Project Description:

Objectives: The objectives of this project are to study: 1) Humoral antibody responses to murine tumors; 2) Cell-mediated immunity to Friend virus-induced leukemia: a) Primary cell-mediated cytotoxic response, b) Secondary cell-mediated cytotoxic response, and c) Generation of cytotoxic lymphocytes *in vitro*; 3) Correlation of *in vitro* immunological reactions to *in vivo* tumor immunity.

Methods Employed: Tumor cells transformed by oncogenic viruses, chemicals, or unknown agents are kept in the tissue culture or as transplanted tumors in mice or rats. The isotopic antiglobulin technique (IAT) was used to perform serological studies. The TSTA was tested by immunorejection experiments.

Major Findings:

I. Humoral Antibody Response to Murine Tumors.

Previous studies showed that transplantable murine tumors (SV-40 tumors and Friend virus-induced leukemia) could produce specific antibody responses to tumor associated antigens. The changes in the level of antibody correlated very well with the status of tumor growth. We have further studied the humoral antibody response of C57BL/6 mice to primary inoculation of Moloney murine sarcoma virus (MSV). Two different stocks of viruses were used: regressor MSV which only produced transient tumor growth, and progressor MSV which gave progressive tumor growth. The kinetics of the antibody response to both virus preparations were biphasic; the level of the antibody first peaked at day 10, declined and became undetectable around day 14, then rose steadily after day 20. In the regressors, the antibody level started to decline after 60 days but the levels remained high in the progressors. The specificity of the antibody responses is under present investigation.

II. Cell-Mediated Immunity to Friend Virus-Induced Leukemia.

The ^{125}IUdR release assay was used extensively to study the cell-mediated cytotoxic responses _in vitro_, and the tumor transplantation test was used to study _in vivo_ tumor immunity.

a. Primary Cell-Mediated Cytotoxic Response.

Previous studies have shown that cell-mediated cytotoxic responses to FBL-3, a syngeneic Friend virus-induced leukemia in C57BL/6 mice, could be demonstrated by the ^{125}IUdR release assay. We have further modified the assay to be performed in Microtest II plates, with harvesting by an automatic harvester (MASH II). This enabled us to perform larger scale experiments. The kinetics of the primary cell-mediated cytotoxic response was biphasic; the reactivity first peaked at days 10-14, declined and became undetectable at days 20-30, and then the reactivity reappeared and persisted up to at least 60 days. The cytotoxic reactivity of both first peak (days 10-14 and second peak (days 40-60) was found to be T-cell dependent. The specificity of the reactivity appeared to be mostly against F (Friend) or FMR type antigens, and correlated well with the specificity seen in _in vivo_ tumor transplantation experiments. The paradox of this kinetic study was the lack of cytotoxic reactivity around days 20-30 at a time when the mice possessed strong _in vivo_ tumor immunity. Various possibilities have been suggested: serum blocking factor, traffic pattern of the circulation of cytotoxic lymphocytes, presence of suppressor cells, circulating soluble antigen, and "homing" of cytotoxic lymphocytes into the site of tumor growth. Our preliminary studies showed that sera from neither progressor nor regressor mice blocked the cytotoxicity; however, a purified γ-globulin preparation would give partial blocking. Soluble antigen extracted from FBL-3 cells (prepared by Dr. Appella, Laboratory of Cell Biology, National Cancer Institute) could completely inhibit the reactivity, whereas a similar preparation obtained from syngeneic spleen cells would not. We have also obtained some evidence for the presence of suppressor cells and for "homing" of cytotoxic lymphocytes into the site of tumor growth.

b. Secondary Cell-Mediated Cytotoxic Responses.

It was of interest to determine whether there was cell-mediated immunologic memory in this system and whether the appearance of cytotoxic lymphocytes could be reinduced by secondary challenge with the tumor cell. Therefore, we have performed extensive studies on the secondary cell-mediated cytotoxic response. It was found that the secondary response peaked earlier (days 3-5), the reactivity was higher and persisted longer than the primary response. The secondary cytotoxic response was also found to be T- cell dependent. The specificity was mostly FMR or F type, with weaker reactivity against a more broadly distributed antigen, consistent with MEV-SA1 described in the MSV system.

c. Correlation of *In Vitro* Immunological Reactions to *In Vivo* Immunity.

1. Time Course of Cell-Mediated Cytotoxic Response Versus Induction of *In Vivo* Tumor Immunity.

Lymphocyte cytotoxicity could be detected as early as 3 days after immunization with FBL-3 cells. In parallel tests of *in vivo* tumor immunity, all mice survived if they were challenged at 3 days or later after such immunization.

2. Adoptive Transfer Experiments.

Although lymphocytes from progressor mice had some cytotoxic reactivity, it was 3-10 times lower than that of cells from regressor mice. When these lymphocytes were used in adoptive transfer experiments, the regressor's lymphocytes gave 90% protection against tumor (FBL-3) challenge, whereas the progressor's lymphocytes only gave 40% protection.

3. Specificity of the Reactions.

Previous serological studies showed that antisera produced against FBL-3 cells could recognize at least three antigenic specificities: F (Friend) type specific, FMR, and broadly cross reactive fetal antigens. *In vivo* tumor transplantation experiments showed that only the F and FMR antigens could function as transplantation antigens. Testing by the ^{125}IUdR release assay, the specificity of the primary cytotoxic response was also mostly against F and FMR antigen, with very little reactivity against non-FMR antigens. When the specificity of the secondary response was studied, the same observations were obtained and this gave very good correlation with the *in vivo* tumor immunity.

The cells which were susceptible to the lymphocytes obtained from FBL-3 immune mice *in vitro* were also immunosensitive *in vivo* and vice versa: i.e., in the ^{125}IUdR assay, FBL-3 or RBL-5 (Rauscher virus-induced) target cells were much more reactive than EL-4 cells (chemically induced); when FBL-3 immune mice were challenged with these different tumor cells, the mice resisted the challenge of FBL-3 and RBL-5 tumor cells, but not EL-4 cells.

Further conditions were obtained by tests of immunogenicity. Previous in vivo tumor transplantation experiments showed that only FMR leukemic cells were able to immunize the C57BL/6 mice against the challenge with FBL-3 cells. Testing by the ^{125}IUdR assay, it was found that only immunization with FMR leukemic cells gave secondary responses after challenge with FBL-3 cells.

Therefore, with several different types of experiments, there was very good correlation between the in vitro immunological reactions and in vivo tumor immunity.

d. Generation of Cytotoxic Lymphocytes In Vitro.

We have been able to generate in vitro both primary and secondary cytotoxic responses of syngeneic lymphocytes against FBL-3 tumor. The cytotoxic lymphocytes were T-cell dependent, and the reactivity was directed mostly against F and FMR antigens. Therefore, these results correlated very well with our previous in vivo immunization studies.

Significance for Biomedical Research and the Program of the National Cancer Institute:

We have continued the study of humoral antibody responses to tumor associated antigens: kinetics, specificity, and relationship to the tumor growth. These studies may be helpful as models for immunodiagnosis of human tumors or for monitoring the course of tumor growth.

An important issue in the field of tumor immunology which remains to be determined is the correlation of the various in vitro immunological reactions to in vivo tumor immunity. Our studies showed that not all antigens detected in vitro could function as transplantation antigens in vivo. However, it now appears that it may be possible to dissect out the various elements in the immune responses to tumor antigens and to determine the role of these various factors in the induction of in vivo tumor immunity.

Proposed Course of Project:

I. Correlation of In Vitro Immunological Reactions to In Vivo Tumor Immunity.

We shall use mainly the ^{125}IUdR assay and the isotopic antiglobulin technique for in vitro study of immunological reactions.

a. Soluble Antigen.

This work will be performed with Dr. Lloyd Law and Dr. E. Appella (Laboratory of Cell Biology, National Cancer Institute). We shall continue to study the effect of soluble antigens in the inhibition of cell-mediated cytotoxicity in vitro, and in the induction of primary and secondary cytotoxic responses, and its correlation to the induction of *in vivo* tumor immunity.

b. Humoral Factors.

More extensive investigations will be performed on the interrelationships of humoral factors, the cell-mediated cytotoxicity, and the *in vivo* tumor immunity. Ascitic fluid, γ-globulin, and sera from progressor and regressor mice will be used for these studies.

II. Humoral Antibody Response to Tumor Associated Antigens.

We shall concentrate on primary tumors (MSV induced and methylcholanthrene induced). The earliest time for antibody detection, the relationship of the kinetics of the antibody response to the tumor growth, and the specificity of the reaction will be studied.

Keyword Descriptors:

Immunological reactions *in vivo*, tumor immunity *in vivo*.

Publications:

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Project No. Z01 CBD 05557-07 LID
1. Laboratory of Immunodiagnosis
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular immune responses in experimental animal tumor systems and in patients with cancer

Previous Serial Number: NCI-5557

Principal Investigator: Dr. Ronald B. Herberman

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Cooperating Units: Viral Leukemia and Lymphoma Branch, NCI; Laboratory of Viral Diseases, NIAID; Laboratory of Microbial Immunity, NIAID; Viral Carcinogenesis Branch, NCI; George Washington University, Litton-Bionetics, Inc., Medical University of South Carolina, Burkitt Tumor Project, Accra, Ghana

Man Years:
Total - 5.00
Professional - 5.00
Other - 0.00

Project Description:

Objectives: The objectives of this project are: (1) to detect tumor specific and tumor associated antigens and quantitative changes in normal antigens on malignant cells; (2) to detect cellular immune reactions to tumor antigens; (3) to evaluate the diagnostic, prognostic and therapeutic significance of cellular immune responses to tumor antigens; (4) to develop and evaluate immunodiagnostic assays for human cancer; (5) to measure general immunological competence of tumor bearing animals and of cancer patients; (6) to detect delayed hypersensitivity reactions and other immunological reactions to human tumor antigens; and (7) to isolate and characterize tumor antigens and other cell surface antigens.

Methods Employed: Lymphoid cells are obtained from tumor-bearing immune control subjects. A variety of normal and tumor cell lines are maintained in tissue culture. Inbred strains of mice and rats, and transplantable tumors induced by viral, chemical or unknown agents are used in model systems. Cellular immune competence is assayed by skin tests; rosette formation;

lymphocyte stimulation by mitogens, antigens and allogeneic cells; and by cytotoxicity reactions. Cellular immune reactions to tumor antigen are measured by skin tests, mixed lymphocyte tumor cell cultures, migration inhibition, and lymphocyte-mediated cytotoxicity assay. Human and animal tumor and normal cell membrane and soluble fractions are prepared for antigenic studies. Subpopulations of lymphoid cells are prepared by separation of rosette-forming cells, passage through anti-immunoglobulin columns, adherence columns, and by antiserum treatments.

Major Findings:

I. Tumor Immunity in Animal Models:

A. MSV Tumor System.

1. Cell-Mediated Cytotoxicity.

Extensive studies have been performed with C57BL/6 mice inoculated with murine sarcoma virus (MSV). Most stocks of MSV cause temporary tumor growth in adult mice and then regression. Stocks of MSV have also been found which reliably produce progressive tumor growth. It has therefore been possible to compare the patterns of cell-mediated immunity in regressors and progressors. With regressor stocks of virus, high cell-mediated cytotoxicity could be detected, in a 4 hour ^{51}Cr release assay, at about 14 days. Reactivity then repidly declined to low levels after 20 days. In progressors, the same kinetics of response were seen but the levels of cytotoxicity were significantly lower.

The kinetics seen in the ^{51}Cr release assay were quite different from reports of some other laboratories, using visual microcytotoxicity assays. We therefore undertook a direct comparison between the ^{51}Cr release assay, an ^{125}IUdR release cytotoxicity assay, and the visual microcytotoxicity assay. Essentially the same kinetics of response were seen in all three assays.

Studies have been performed to determine the nature of the effector cells in these cytotoxicity assays. The ^{51}Cr release assay was shown to be entirely dependent on T-cells, with reactivity eliminated by treatment with anti-θ plus complement. Removal of macrophages had no effect. A paradoxical finding has been that a majority of the cytotoxic cells were consistently retained on Sephadex-anti-gamma globulin columns and recovered in the "B cell" fraction. It seems likely that the cytotoxic cells are T-cells with some immunoglobulin on their surface. These findings are consistent with other observations that activated T-cells may have surface receptors for immunoglobulins.

Extensive studies have been performed on the cryopreservation of effector cells and of target cells in this system. This has proven extremely useful in standardizing the assay and minimizing day-to-day variations. This has also allowed us to rapidly detect changes in the antigenicity and susceptibility to lysis of fresh target cells.

An apparent paradox in the MSV tumor system and other syngeneic systems has been the short-lived cytotoxic cellular immune response, in contrast to the long lasting in vivo resistance to tumor challenge. We have recently demonstrated that challenge with tumor cells carrying the appropriate antigens, even many months after primary inoculation with MSV, resulted in rapid appearance of a secondary cytotoxic response. High levels of cytotoxic reactivity could be consistently detected, mainly in the region of tumor cell challenge.

A competitive inhibition assay has been developed to analyze the specificity of the ^{51}Cr release cytotoxicity assay. Studies of the MSV system have indicated that the antigens detected were not directly related to MSV or FMR leukemia antigens, but rather to mouse endogenous type C viruses. Many mouse tumor cell lines in vivo, and particularly in vitro, induced by a variety of methods or agents, have been shown to contain this antigen, designated MEV-SA1, and other markers of endogenous viruses. Infectious endogenous viruses could be isolated from most of these cells and these could infect mouse embryo fibroblasts or xenogeneic cultured cells. Upon infection of these cells with the endogenous viruses, expression of MEV-SA1 could be detected, confirming the direct association of this antigen with mouse endogenous type C viruses.

2. Lymphocyte Stimulation and In Vitro Generation of Cytotoxic Cells.

Cell-mediated immunity could also be detected in this system by the lymphocyte stimulation assay. Low levels of stimulation were seen when lymphocytes from mice inoculated 14 days before with MSV were cultured with mitomycin treated Rauscher leukemia cells. Incubation of immune lymphoid cells with tumor cells also resulted in the generation of an in vitro secondary cytotoxic response. Strong cytotoxicity could be demonstrated, even at low effector:target cell ratios. Suppressor cells in the spleens of these tumor bearing mice were shown to partially inhibit this stimulation by tumor cells, inhibited the in vitro generation of cytotoxic cells, and also depressed reactivity to mitogens and to allogeneic cells. Extensive studies have been performed to characterize the mechanism of this suppression and the nature of the cells mediating these effects. Considerably evidence has indicated that macrophages are responsible for the suppression. These suppressor macrophages were found to have a variety of effects. In addition to depressing proliferation of lymphocytes, they have also been shown to have potent inhibitory effects on the in vitro growth of tumor cells. These growth inhibitory effects were nonspecific, in that a wide variety of tumor cells were equally affected. Potent suppressor cells could be induced not only by MSV tumor growth, but also by growth of a variety of other mouse tumors and by inoculation of Corynebacterium parvum.

In addition to suppressor macrophages, it was found that some tumor cells themselves could inhibit lymphocyte stimulation reactions. This tumor cell-mediated depression has been found to be mediated by cell-free filtrates derived from the tumor cells. Current evidence suggests that it is due to an infectious agent, presumably a virus; inhibitory activity is sedimentable at 100,000g, is inactivated by ultraviolet irradiation, and can be neutralized by ascites fluid from mice carrying the tumors. Both the suppressor cells and the inhibitory factors within tumors may account for some of the immunodepression associated with tumors, and may also explain many of the negative cellular immunity reactions seen in tumor systems [e.g. see below in the (C58NT)D rat tumor system].

3. Effector Cells Present Within Tumors.

The direct *in vivo* interaction of effector cells with tumor cells would seem to be the most relevant issue in host resistance against tumor growth. It was therefore of considerable interest to look for effector cells within primary MSV tumors. In collaborative studies with Dr. Steven Haskill (Medical University of South Carolina), tumors were dissociated and cells separated on 1g density gradients. Highly reactive effector cells in the ^{51}Cr release cytotoxicity assay were found, and also active suppressor macrophages. The size characteristics of the cytotoxic cells in the tumor were somewhat different from those found in the spleen at the same time.

4. Adoptive Transfer of Immunity.

Of central importance in studies of tumor immunity is the relationship of the results obtained in *in vitro* assays to the *in vivo* response to tumor growth. An adoptive transfer system has, therefore, been established to determine the effect of various cell populations, at various times after immunization, in resistance against tumor growth. Cells from regressor mice, from 14-40 days after MSV inoculation, protected recipients completely from MSV tumor induction. Cells at 14 days after MSV also protected very well against challenge with a Rauscher virus-induced tumor. Cells from progressor mice at 14 days also gave some protection against MSV and tumor cells. It should be noted that these cells were taken at the peak of the *in vitro* cytotoxic response. The *in vivo* effects of suppressor cells are also currently being investigated in this system.

B. Gross Virus-Induced Leukemia, (C58NT)D, in Rats.

1. Lymphocyte Stimulation.

In earlier studies, we demonstrated that inoculation of this tumor into syngeneic rats produced strong cytotoxicity in the ^{51}Cr release assay at 10 days, with a rapid decline in reactivity thereafter. In contrast, the lymphocyte stimulation assay with mitomycin treated tumor cells first became positive at 15 days and peaked at 25-40 days in rats which regressed their tumors. With progressive tumor growth, no positive stimulation was seen. These tumor bearing rats were also found to have depressed responses to mitogens. As with the MSV system in mice, this lack of stimulation in progressor rats was found to be due to suppressor cells.

After removal of suppressor cells on adherence columns, positive stimulation by tumor cells could be detected. In addition to the suppressor cells, high concentrations of (C58NT)D tumor cells themselves were shown to inhibit lymphocyte stimulation. This effect seems to be the same as that described above for mouse tumor cells.

2. Secondary Cytotoxic Responses.

As in the MSV tumor system in mice, re-challenge of previously inoculated rats with tumor cells resulted in the rapid appearance of cytotoxic reactivity. In contrast to the mouse, this secondary response was more generalized, with strong activity in the spleen as well as in the region of tumor challenge. In vitro incubation of immune cells with tumor cells also resulted in a brisk secondary cytotoxic response.

3. Mitogen-Induced Cytotoxicity.

In addition to specifically immune cytotoxic cells, potent lytic effects against (C58NT)D could be seen with normal lymphocytes after incubation with phytohemagglutinin (PHA) or endotoxin (LPS). The PHA induced reactivity was shown to be mediated by T-cells, whereas the LPS induced reactivity was mediated by non T-cells, perhaps B cells. The LPS effects were of particular interest, since, in contrast to the mouse, it has been extremely difficult to demonstrate stimulation of rat lymphocytes by LPS.

C. Natural Cell-Mediated Cytotoxicity Against Tumor Cells.

Lymphoid cells from many normal rats and mice, of a variety of inbred and random bred strains, were found to have reactivity, in the ^{51}Cr release cytotoxicity assay, against several syngeneic and allogeneic tumors. In both rats and mice, peak reactivity was seen in young animals, usually between 5-8 weeks of age. Older animals had little or no demonstrable reactivity. In the rats, this activity could be shown to be independent of T-cells and macrophages. The characteristics of the reactivity in the mouse has been studied in depth. Very high reactivity was seen with cells from athymic, nude mice, suggesting that the mouse natural reactivity was also independent of T-cells. In addition, treatment of cells with anti-Θ plus complement had little effect, and removal of macrophages also did not inhibit reactivity. No cell surface markers could be found on the natural cytotoxic cells, and it has been concluded they represent a novel subpopulation of lymphoid cells, tentatively designated N cells. Target cells susceptible to lysis included tumors induced by oncogenic type C viruses but also tumors induced by other means and expressing endogenous type C viruses. By use of the inhibition assay, the natural cytotoxic reactivity in both mice and rats could be shown to be related to expression of endogenous type C viruses, but the specificities were distinct from those recognized by cells from deliberately immunized animals.

II. Cell-Mediated Immunity in Patients with Cancer.

A. Cellular Immune Competence.

A series of assays for measuring the general cell-mediated immune competence of cancer patients have been established in the Laboratory. One of these is the enumeration of the percent of T-cells in the peripheral blood by rosette formation with sheep erythrocytes. It was found that a number of variables affected the percentage of rosette forming cells (RFC). Assays performed under suboptimal conditions, at 29°C, were found to be particularly discriminatory between cells from cancer patients and cells from controls. A large proportion of patients with carcinomas, sarcomas, and melanoma were found to have significantly depressed levels of RFC in this assay. Both patients with localized disease (77%) and those with metastatic disease (100%) had levels of RFC significantly below those of normal controls. In contrast, only 10% of normal individuals had significantly depressed percentages of RFC. This assay is still under investigation regarding its potential application to the immunodiagnosis of cancer, and the monitoring of the clinical course of disease in cancer patients. Studies are also being performed to determine the correlation between %RFC and functional activity in assays of cell-mediated immunity.

An additional, intriguing observation made with the rosette assays has been that malignant pleural effusions contained high percentages of RFC, in the face of depressed levels in the peripheral blood. The significance of this consistent observation is actively being investigated.

The ability of human T-cells to form rosettes with sheep erythrocytes has also led to a very useful method for isolation of T-cells from peripheral blood leukocytes. Fractions with high yield and purity have been obtained. In assays of lymphocyte stimulation, reactivity to the mitogens PHA and concanavalin A and to the antigens PPD and tetanus, and to allogeneic leukocytes has been completely restricted to the T-cell fraction. Different results were seen with a staphylococcal antigen; this produced strong stimulation of non-T-cells as well as T-cells. It seems likely that this antigen represents one of the final strong stimulants for human B cells or other non-T-cells. In assays of cytotoxicity against human lymphoid cell lines, both T and non-T-cells were also found to be reactive.

B. Cell-Mediated Immunity to Human Tumor Associated Antigens.

1. Leukocyte Migration Inhibition.

Further studies have been performed with the direct leukocyte migration inhibition assay. The leukocytes of patients with carcinoma of the breast, malignant melanoma, carcinoma of the lung, and Ewing's sarcoma have been shown to be specifically inhibited by 3M KCl extracts of autologous and allogeneic tumors of the same histological type. Little cross-reactivity of patients with other types of cancer or of normal controls has been seen. During the past year, a major emphasis has been placed on the standardization of the assay and on modification in technical factors to improve sensitivity and reduce variability. One concern has been to identify large, standard tumor extracts which could be used repeatedly for monitoring of cancer patients. One possible source, metastatic tumor deposits, has not yielded good activity. In contrast, extracts from malignant pleural effusions of breast cancer and of lung cancer have been very active, giving a high percentage of specific, positive reactions. A variety of established tissue culture lines derived from tumors are being carefully evaluated for antigenicity. Several lines have already been identified which are antigenic. These offer the potential for preparation of very large, standard antigen preparations.

It has recently been reported that some patients with breast carcinoma have specific cell-mediated immunity to antigens associated with mouse mammary tumor virus (MTV). We have performed a series of experiments to evaluate this. We have indeed found that a high proportion of breast cancer patients, and not normal individuals or other cancer patients, react with MTV and not with control mouse materials. Studies are being actively pursued to determine the significance of this observation.

The direct leukocyte migration assay has some important disadvantages. It requires large numbers of leukocytes and antigen, and is dependent on the functional activity of the patient's granulocytes for migration. Efforts in the past year to overcome these problems have yielded encouraging results. It has been possible to set up the assay in agrose droplets, which requires only 10% of the cells and antigen used in the standard, capillary tube assay. In addition, it has been possible to perform indirect assays of migration inhibition. Antigenic extracts were first incubated with the patient's cells, and then supernatant fluids containing the inhibitory factors were added to normal granulocytes for assay. This modification in the assay offers the potential for repeated testing of the same supernatant, and also simultaneous testing of supernatants generated from a given patient at different times in the course of disease, allowing a more accurate assessment of changes in reactivity.

2. Lymphocyte Stimulation by Tumor Cells and Extracts.

In earlier studies, we found that allogeneic extracts of tumors were not useful for study of stimulation by tumor associated antigens, since normal alloantigens could also be recognized in these extracts. In the past year, a major effort has been made to study stimulation of cancer patients' lymphocytes by autologous tumor cells and by membrane extracts and 3M KCl extracts of autologous tumors. In approximately 50% of the patients tested thus far, stimulation has been seen with tumor materials and not with normal control tissues. The membrane extracts appeared particularly promising, in that very low concentrations gave positive results.

Separation studies of the lymphocytes responsible for this activity have been initiated. Thus far, T-cells of cancer patients, separated by rosetting, have been found to be reactive. In contrast, with a patient with a benign giant nevus, non-T-cells were responsible for the reactivity.

Patients with cancer have been shown to have delayed hypersensitivity reactions to extracts of tumor cells and of some tissue culture cell lines derived from tumors. A prospective study of reactivity of African patients with Burkitt's tumor is being performed with extracts from a cell line derived from a Burkitt tumor. This study has now been in progress for over a year and the results are quite interesting. Reactivity to this extract has correlated well with clinical status. Before therapy, only patients with small localized tumors have reacted. When patients have been successfully treated and then tested in remission, a high proportion have been reactive. Patients failing to go into remission, or patients with early relapse have tended to be unreactive, although they were not anergic. The skin reactivity has been correlated with serological studies of antibodies to EBV associated antigens, and a very significant inverse correlation between skin reactivity to the cell line extract and antibodies to EBV nuclear antigen has been found.

Efforts have been made to solubilize and isolate the skin reactive antigens from some tumors. In malignant melanoma, it has been possible to prepare semi-purified antigens which give a high proportion of reactions in melanoma patients and little reactivity in patients with other types of cancer. The possible diagnostic potential of this antigen is being evaluated in patients suspected of having ocular melanoma. Thus far, skin tests with this antigen have been able to accurately distinguish patients with ocular melanoma from those with benign, simulating lesions of the eye or with metastatic cancer in the eye. The separated melanoma antigens have also been found to give positive reactions in the leukocyte migration assay, so that it may be possible to perform more extensive tests *in vitro* to evaluate the diagnostic potential of this reactivity.

Significance to Biomedical Research and the Program of the Institute:

Detection of tumor antigens and of immune reactions to tumor specific antigens is essential for progress in tumor immunology. The sensitive assays for humoral and cell-mediated immunity which have been developed in the laboratory have already proved useful in studying the immune response to animal and human tumors.

The ability of our laboratory to perform multiple assays of humoral and cell-mediated immunity to tumor antigens should allow detailed dissection of the role of the immune response in limiting or preventing tumor growth. These assays may also be useful in immunodiagnosis of cancer and in measuring changes in immunity, in response to chemotherapy or immunotherapy.

The available immunological techniques have also allowed progress in the solubilization and isolation of tumor associated cell surface antigens. Such studies will permit a comparison of the physicochemical nature of tumor antigens and normal histocompatibility antigens and may provide for active immunization of animals and tumor patients against their tumors.

Proposed Course of Project:

Studies are in progress to further understand the immune responses of experimental animals and of patients to tumor associated antigens.

In experimental animals, further studies will be performed on the nature of the cells involved in various immune fractions and on their mechanism of action. Studies on the interrelationships between the different assays will be continued. Of particular emphasis will be the search for possible differences in the specificity of the antigens detected in each of the assays, and on the role of each of the *in vitro* reactivities for *in vivo* protection against growth of tumors. The latter question will be examined by the extensive use of adoptive transfer experiments.

Studies of cell-mediated immunity in cancer patients will be continued. Assays of general immunological competence and of reactivity to tumor associated antigens will be used to monitor the response of patients to immunotherapy with BCG or with tumor cells. The potential of each of these assays for the immunodiagnosis of cancer will be carefully examined. Studies on the interrelationships between the various assays of reactivity to tumor antigens will be continued. Attempts will be made to better understand the specificity of the antigens detected in each of these assays. The results of each assay will be correlated with clinical parameters to determine which assays are most useful for prognosis.

During the next year, a major new emphasis will be made on isolation of the tumor associated antigens reactive in some of the assays of cell-mediated immunity. Antibodies will be prepared against these antigens and assays will be developed to evaluate the immunodiagnostic usefulness of these reagents.

Keyword Descriptors:

Cell-mediated cytotoxicity, tumor immunity, lymphocyte stimulation, rosette forming, mitogen induced cytotoxicity

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LABORATORY OF CELL BIOLOGY

SUMMARY

July 1, 1974 - June 30, 1975

Selected highlights of achievements in the Laboratory of Cell Biology

Solubilization and purification studies with TSTA and histocompatibility (H-2) cell surface antigens continue.

Histocompatibility Antigens (H-2 and HL-A)

Spleen cell membranes ($H-2^a$) have been solubilized with limited papain digestion and further purified through the use of DEAE chromatography, on lectin bound Sepharose and G-100 superfine Sephadex columns. As determined using a radioimmunoassay and inhibition of cytotoxicity against monospecific antisera these preparations are now 75-80% purified. Limited biologic assays are now being done with analytical amounts of these purified materials. Also using these highly purified preparations ($H-2^a$ haplotype) it was shown that the antigen molecules consist of fragments: 37,000 daltons and 11,000 daltons. The 37,000 fragment contained H-2 allospecification and the small component (11,000 daltons) that was completely purified is the β-2 microglobulin - a homolog of the β-2 microglobulin (HL-A) of man.

Recent reports have demonstrated that papain-solubilized, highly purified HL-A antigen molecules are formed of 2 components of dissimilar size bound non-covalently: the 11,000 dalton component is human β-2 microglobulin, the 34,000 dalton component contains the alloantigenic determinants. The large component has been prepared in the native state by dissociation with anti- β2 microglobulin, by dissociation at acid pH; in these cases loss of alloantigenic determinants occurs. Large fragment preparations containing mostly HL-A2 or HL-A7 were obtained from RPMI 1788 cells and compared for their physico-chemical properties. It was found that the large fragments were all of the same molecular size; differences however in charge were found using electrofocusing on urea acrylamide gels. Comparison of total amino acid composition showed small but significant differences between the 2 preparations. Attempts are being made to determine the number of carboxymethylcysteine peptides in these preparations in view of the possible evolutionary relationship of the constant region of immunoglobulin heavy chains to histocompatibility antigens.

The 11,000 dalton fragment of the $H-2^a$ antigen molecule was found to be extractable by 3M NaSCN and then purified by successive chromatography and electrophoresis to complete homogeneity. Total amino acid composition has revealed homology with rat and human fragments of β-2 microglobulin. Antigenically this cross reacts with rabbit antibodies directed against the rat Ag-B 11,000 dalton fragment and human HL-A fragment.

Tumor Specific Antigens

The methods used for further purification of H-2 antigens has been adapted for TSTA of both the DNA virus- and RNA virus-induced neoplasms: mKSA(SV40) and RBL-5(RLV). Materials have been recovered with a high degree of purity. In the case of purified TSTA from the mKSA sarcoma as low a concentration of 5 μg total protein was found capable of inducing tumor rejection (TD 80-90) of the syngeneic tumor; specific lymphocyte stimulation was also achieved with optimal concentrations of TSTA of 1.0 μg/well (5.0 μg/ml).

Previous work with a chromatographed fraction of TSTA from RBL-5 leukemic cells strongly indicated that this cell-membrane located tumor antigen was distinct from infectious virus and from virion components though presumably virus specified. Syngeneic antisera, not containing neutralizing activity detected virion antigens in our solubilized preps but there also existed another component with strong biologic activity that we presumed was TSTA. We now have at our disposal P-30 and GP-70 virion components and their respective antisera. These components located on the cell membrane give indication of biologic activity in certain *in vitro* assays. We are now in a position to be able to detect their presence on RBL-5 cells and to look for activity in *in vivo* tumor rejection thus implicating or ruling out these components in *in vivo* tumor rejection.

RBL-3, another tumor induced by Rauscher-MuLV (RLV) in the same manner as RBL-5, and carried in *in vitro* culture, was found to be a non-producer in that no infectious RLV was detectable. This cell line is currently being studied intensively. The cultured cells were immunogenic in C57BL/6 (B6) mice against challenge with FBL-3, a Friend-MuLV-induced tumor, and immunization of B6 mice with the latter induced an immunity against challenge with RBL-3 tumor (adapted back to *in vivo* ascites growth from the culture). Furthermore, immunization of B6 mice with MSV(RLV) induced resistance against RBL-3 challenge. These facts establish that RBL-3 cells have FMR group cell surface antigen (TSTA type). By a complement fixation test this RBL-3 culture was shown to possess *gs* antigen. The presence and possible function of envelope antigen is to be investigated. Crude membranes are being prepared from these cells for immunization and solubilization.

A micro mixed lymphocyte-tumor cell assay employing thymidine incorporation has been developed in collaboration with Litton Bionetics. This assay has been shown to be specific in causing stimulation of sensitized lymphocytes by tumor cells of the SV40-induced sarcoma mKSA. Soluble antigen (SA) can be substituted for tumor cells and as little as 0.1 μg or optimally 1 μg (SA) induced significant lymphocyte stimulation. This assay is one method now being used to monitor further fractionation and purification of this TSTA.

It was reported previously that we were able to clone neoplastic cells from an MSV(MLV)-induced hemangiosarcoma (XM-1). These clones revealed a heterogeneity as regards their virus production (MLV), presence of defective MSV, transplantability and presence of TSTA. We have continued studies with one of these clones, H-11, adapted to tissue culture growth. This clone continues to show no evidence of infectious virus as determined by sensitive plaque - or focus-forming assays and by the buoyant density gradient assay. MSV is not rescuable yet this clone retains a moderately strong TSTA that is MSV(MLV) specific. We are presently further characterizing this TSTA.

TSTA of plasma cell (PC) tumors have been studied in BALB/c mice. One group of PC, induced by mineral oil was shown to have either individual or in some cases, cross-reacting TSTA. A second group of PC induced by Abelson virus (A-MLV) were shown to have only cross-reacting antigens within the group but not with the TSTA of mineral oil-induced PC. These A-MLV induced PC were producers of C-type virus - thus virion structural components probably are the effective antigens. Since viral structural components (P30, P15, GP 69/71) and antisera to these are now available, we are in a position to distinguish between the role of structural virion components and virus-specified new cellular antigens in tumor rejection, in in vitro cytotoxicity and the relationship between in vivo and in vitro events.

Studies continue on the usefulness of the alpha-fetoprotein (AFP) marker in human disease: (1) AFP and serum chorionic gonadotropin (hCG) markers have been used as a prospective manner to gauge effectiveness of treatment in hepatocellular carcinoma and embryonal cell testicular carcinoma. (2) AFP levels are high in some forms of tissue damage and appear to be related to liver degeneration. (3) A reference standard has been developed for laboratories wishing to quantitate AFP.

D1-murine leukemia virus (MuLV), an N-tropic type C virus, was isolated from a spontaneous reticulum cell neoplasm, type B (RCN-B) of an SJL/J mouse. Its distinctive type-specific envelope antigen and ability to induce a lymphocytic leukemia in SJL/J mice injected as newborns have been described. However, no RCN-B neoplasms have been induced to date.

BALB/c mice were used to determine the pathogenic potential of D1-MuLV. When injected into BALB/c mice 1 day after neonatal thymectomy, 30% of the inoculated mice developed RCN-B in 5 months, whereas none of the uninoculated controls did. From the spleen and lymph node extracts of all RCN-B-bearing mice tested, B-tropic type C viruses (designated E1-MuLV) were isolated in high titers (10^5 - 10^6 XC-plaque forming units/ml). Only low titers (10^1 - 10^2 XC-plaque forming units/ml) of N- or B- tropic viruses were isolated from those thymectomized mice inoculated but non-tumorous, whereas only N-tropic viruses were detected in the uninoculated thymectomized mice. No virus was isolated from the nonthymectomized, untreated controls. Antigenically, the viral envelope antigen (VEA) of E1-MuLV was distinct from those of D1-MuLV, x-VEA, or Gross-VEA, but some cross-reaction with AKR-MuLV-VEA was observed. For the following reasons it was suggested that D1-MuLV was specifically related to RCN-B oncogenesis in thymectomized

BALB/c mice, and that El-MuLV was selectively activated in RCN-B cells. The importance of this observation lies in the fact that a covert endogenous virus may be activated in the neoplastic cells by inoculation of another infectious type-C virus.

Through the use of high resolution electron microscopy and a combination of preparative techniques, it has been possible to gain important new information about the structure of intracisternal A particles (IAP). Up to this point all of the studies of IAP have involved either thin sectioning or negative staining. The results reflect essentially what is seen in a one-dimensional plane. Through the use of critical point drying plus platinum shadowing it has been possible to view unsectioned IAP as "whole mount" preparations with three dimensions. Further, latex spheres were added to purified IAP as an internal marker for measurements and for confirmation of particle configuration. Our data indicate that isolated IAP have an average diameter of 85 nanometers, they are essentially uniform spheres as indicated by the shadow length, and they consist of two concentric shells surrounding a hollow core. It can be demonstrated that numerous single IAP have an apparently intact outer shell. This is an important finding because previous reports have suggested that the maturational defect in IAP is a failure of particle buds to be released from the membranes of endoplasmic reticulum. The fact that we can isolate IAP that do not exhibit defects in the outer shell strongly suggests that a certain proportion of these particles are released from the membrane into the cisternal spaces of the endoplasmic reticulum. These results are buttressed by the fact that the conditions of isolation preclude any synthesis or repair of defects in the outer shell of the particle.

A detailed description of the appearance of IAP during early embryogenesis has been made. In tracing the morphologic sequence of events preceding the development of IAP, it was discovered that crystalloid aggregates contiguous with the endoplasmic reticulum appear first in the fertilized egg. These structures increase in size and number until the morula stage. During the blastocyst stage crystalloid units often form densely staining plaques. As these crystalloids continue to develop, budding and complete IAP begin to appear. By the time the embryo reaches the outgrowth stage these crystalloids and plaques can no longer be detected, although IAP can still be found. Through the use of immunoelectron microscopy, it may be possible to determine whether or not these crystalloids and IAP share common antigens.

A study of surface receptors on human mononuclear leukocytes (HML) continues. It is known that HML possess IgG surface receptors which may be involved in phagocytosis. Such receptors can be detected by the ability of HML to form rosettes with IgG-coated erythrocytes. During phagocytosis this IgG surface receptor activity disappears. Through the use of a chemotactic factor derived from human complement, it is possible to demonstrate that HML possess a definite polarity in their response to such a stimulus. When HML are exposed to a chemotactic gradient across a millipore filter, these cells line up at the filter surface and long pseudopods are thrust into the filter pores. In this condition the tail of the HML cell farthest from the chemotactic stimulus does not form rosettes with IgG coated erythrocytes. This loss of receptor activity is not due to inhibition by chemotactic factor since exposure of cells to this factor on both sides of the filter chamber does not cause similar impairment of rosette formation. In summary, the results indicated that HML lose their IgG binding receptors at the trailing end of the cell in response to a chemotactic stimulus. The mechanism by which these changes occur remains to be determined. It could reflect either internalization, rearrangement of migration of receptors or a combination of such events.

Groups of BALB/c myeloma proteins that bind phosphorylcholine, β1→6D galactan, α1→3, α1→6 dextran, β2→6 fructosan, DNP have been collected and studied both structurally and antigenically.

Structural studies involve determination of primary structures and 3-dimensional structures by X-ray crystallography. A 3.1 Å electron density map of the phosphorylcholine binding myeloma protein McPC 603, based on the complete sequence of the V_H and 80% of the V_L has been completed. The crystal was made with the ligand in site and this permits the definition of the binding site. A second crystal is being prepared with J 539. This information provides the means for a new approach to immunochemistry.

Essentially, the Ig combining site is a localized at one pole of the Fv, in a continuous surface bounded by the hypervariable segments from the V_L and V_H. Since the internal architecture of the V_L and V_H domains in both mouse and man is extremely similar, by knowing the 3-dimensional structure of the binding site it will become possible to modify the structure of antibodies.

We are continuing to screen for new antigen binding myeloma proteins and test each for their ability to crystallize.

In the last year the levan binding myeloma protein system has been intensively investigated. Fourteen proteins have been collected and characterized. These have been divided into 2 groups: a large group of proteins that bind β2,1 linked fructosans and a smaller group with specificity for β2,6 linked fructosans. The amino acid sequences are being determined.

We have studied the individual and cross-specific idiotypes of these proteins. Each protein has an individual idiotype and one or more cross specific determinants. The large number of these determinants suggests and the preliminary sequence data indicate a degree of variability of closely related proteins that may be the result of a form of somatic variation. We are actively pursuing these studies.

Many of the homogeneous immunoglobulins of plasmacytomas of BALB/c mice have been shown to demonstrate antigen binding activity and in fact groups of proteins have been characterized which bind the same antigen. We have been studying three of these groups of proteins in an attempt to 1) determine the structure of the antibody combining site, and the interaction between hapten and antibody at the molecular level and 2) ascertain the type of diversity that may occur among a group of proteins which bind the same well defined hapten.

The crystallization of the Fab fragment from the phosphorylcholine binding myeloma protein McPC603 and its general structure at 4.5 Å resolution have been previously described. As a result of the determination of the variable region amino acid sequence and the further refinement of X-ray data, a high resolution model of the antibody combining site has been constructed. We have recently reported a detailed description of the combining site and the detailed interactions between hapten and protein. A cleft has been found at the tip of the molecule which is formed by the three hypervariable regions of the heavy chain and two of the three from the light chain. This surface, composed of the immunoglobulin hypervariable regions, seems to be universal among antibodies and thus provides the structural basis for antibody function and homology. The hapten, phosphorylcholine has been found to form hydrogen bonds with a tyrosine residue in the first heavy chain hypervariable region and an arginine in the second heavy chain hypervariable region. Other interactions have also been observed with other portions of the hypervariable surface. Data refinement is continuing to further clarify some portions of the structure and to complete analysis of the constant region. Similar studies are continuing on the crystallized Fab fragments of J539, a galactan binding protein, to permit the detailed comparison of two proteins with different antigen binding specificities.

Preliminary comparative sequencing studies of five phosphorylcholine binding myeloma proteins have previously been reported. These studies have been extended and the heavy chain variable region determinations on all of the proteins have been completed. This comparison has permitted an assessment of the degree of variability among proteins demonstrating the same hapten binding specificity. The differences observed among these proteins have also provided a unique opportunity to assess the possible mechanism involved in the generation of antibody diversity. Studies on the light chains of these proteins are continuing.

There has been discovered a structural basis for the tumor specific Phe-tRNA found in three rat hepatomas but not in normal adult, newborn, fetal or embryonic liver. Several chemical and immunological studies indicate that this hepatoma associated Phe-tRNA lacks the fluorescent Y base which is characteristic of all eukaryotic Phe-tRNAs. Present studies concern purifying a large amount of this tRNA for sequence studies which will indicate whether the missing Y base is the only difference from normal or whether other bases are different also, which would indicate that a new tRNA gene is transcribed in these tumors.

During the last year we have examined the factors which influence protein-protein interactions. In the case of L-asparaginase from E. coli, we found that the presence of the substrate, L-asparagine, or its D-isomer, retard dissociation of the tetrameric enzyme and also enhance the rate at which the denatured subunits reassemble. The nature of the solvent also influences subunit interactions as observed with a homologous series of polyhydroxy compounds ranging from ethylene glycol to inositol. Replacement of water of the solvent with these hydrophilic solvents also influences interaction of tetrameric units of sickle cell hemoglobin.

The lower numbers of the series prevent aggregation while the polyhydroxy compounds with more than four hydroxymethyl groups promote association to the polymer.

Direct modification of cell surface carbohydrates was achieved by certain metabolic modifiers of the biosynthetic pathways of glycoproteins and glycolipids. Such modifiers as the fluorinated N-acetyl-glucosamine, closely resembling N-acetyl-glucosamine a common metabolic intermediate, were studied. Replacement of hydrogen by fluorine results in little steric change, but the vastly different electronegativity has a definite effect on the interaction of a fluorine containing molecule with an enzyme. It was found that in extracts of mouse cells addition of non-toxic amounts (.05mM) of the fluorinated N-acetyl-glucosamine, the enzyme dependent synthesis of the nucleotide sugars uridine N-acetyl-glucosamine pyrophosphate and cytidine-acetylneuraminiyl monophosphate was greatly inhibited. These neucleotide sugars are key intermediates in sialic acid biosynthesis. This method allows establishment of a low steady pool of precursors, thus an irreversible reduction in sialic acids at the cell surface. Enzymic modifications such as by neuraminidase are found to be only transitory, as cells repair their surface. The metabolic method and the clarification of the mechanism opens important new ways to study the biologic consequences of alteration of sialic acid residues in the highly hydrophilic glycoproteins on the outermost cell surface. The role of this residue on in vitro and in vivo characteristics of malignant cells is being investigated.

Project No. Z01 CBD 03200-07 LCBGY
1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Factors influencing the induction and repression of primary tumors and the growth and inhibition of transplantable neoplasms. (Host and tumor cell variables)

Previous Serial Number: NCI-3200

Principal Investigator: Dr. Lloyd W. Law

Other Investigators:
Dr. E. Appella (Z01 CBD 03229-07 LCBGY)
Dr. Kenneth Chang (Z01 CBD 05550-07 LCBGY)
Dr. M. Rogers (Z01 CBD 03200-07 LCBGY)
Dr. Ole Henriksen (Z01 CBD 03200-07 LCBGY)
Dr. Barry Fernbach (Z01 CBD 03200-07 LCBGY)
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Dr. J. McCoy (Litton Bionetics)
Dr. Jack Dean (Litton Bionetics)
Dr. Robert Ting (Biotech.)

Man Years:
Total - 2.5
Professional - 6.5
Other - 9.0

Project Description:

Objectives: Studies of tumor antigens and the immune responses they invoke; studies of the biological properties of solubilized, purified histocompatibility antigens, and of tumor antigens of the transplantation type - those specified by viruses (VSTA) and those induced by chemical carcinogens (TSTA).

Histocompatibility Antigens

We are now in the process of assaying biologically purified H-2 antigens of the $H-2^a$, $H-2^b$ and $H-2^d$ haplotypes. Spleen cell membranes that have been solubilized with limited papain digestion have now been further purified through the use of DEAE chromatography, on lectin-bound Sepharose and G-100 superfine Sephadex columns. As determined using a radioimmunoassay and inhibition of cytotoxicity these preparations are now 75-80% purified. The immunogenic and tolerogenic potentials of these materials are now being assayed. The highly purified $H-2^a$ haplotype materials have been shown to consist of 2 fragments of MW

37000 daltons and 11000 daltons. The small fragment identified as β-2 microglobulin is now highly purified and its biologic features may now be examined as well as the biologic characteristics of the 37000 dalton fragment separate from β-2 microglobulin. It is clear that amino acid composition and sequencing of H antigens is now closer to reality.

Studies of the lesion responsible for the induction of humoral tolerance in congenic B10.D2 mice induced by soluble $H-2^a$ antigen have revealed that both T-derived and B-derived cells are tolerized. Whereas complete operational tolerance is not achieved in the direction B10.A → B10.D2 we now know that ~ 20% of B10.A congenic mice are rendered completely tolerant to B10.D2 papain-solubilized material. It is thus clear that a severe modification of the structure of the $H-2^a$ molecule is not the sole reason for the characteristic humoral tolerance only that is induced with this material.

Tumor Specific Antigens

The methods used for further purification of H-2 antigens have been adapted for TSTA of both the DNA virus- and RNA virus-induced neoplasms: mKSA(SV40) and RBL-5(RLV). Soluble materials have been recovered with a high degree of purity. In the case of purified TSTA from the mKSA sarcoma as low a concentration of 5 μg total protein was found capable of inducing tumor rejection at a cell concentration of TD 80-90 of the syngeneic tumor; specific lymphocyte stimulation was also achieved with optimal concentrations of TSTA of 1.0 μg/well (5.0 μg/ml).

Previous work with a chromatographed fraction of TSTA from RBL-5 leukemic cells strongly indicated that this cell-membrane located tumor antigen was distinct from infectious virus and from virion components though presumably virus specified. Syngeneic antisera, not containing neutralizing activity detected virion antigens in our solubilized preps but there also existed another component with strong biologic activity that we presumed was TSTA. We now have at our disposal P-30 and GP-70 virion components and their respective antisera. These components located on the cell membrane give indication of biologic activity in certain *in vitro* assays. We are now in a position to be able to detect their presence on RBL-5 cells and in solubilized preps and to look for activity in *in vivo* tumor rejection thus implicating or ruling out these components in *in vivo* tumor rejection.

A degree of purification similar to H-2 has now been achieved using these membrane-bound TSTA. Of interest is the fact that both mKSA and RBL-5 antigens co-chromatograph with or very near H-2 suggesting some identity perhaps.

RBL-3, another tumor induced by Rauscher-MuLV (RLV) in the same manner as RBL-5, and carried in in vitro culture, was found to be a non-producer in that no infectious RLV was detectable. This cell line is currently being studied intensively. The cultured cells were immunogenic in C57BL/6 (B6) mice against challenge with FBL-3, a Friend-MuLV-induced tumor, and immunization of B6 mice with the latter induced an immunity against challenge with RBL-3 tumor (adapted back to in vivo ascites growth form the culture). Furthermore, immunization of B6 mice with MSV(RLV) induced resistance against RBL-3 challenge. These facts establish that RBL-3 cells have FMR group cell surface antigen (TSTA type). By a microcomplement fixation test this RBL-3 culture was shown to possess gs antigen. The presence and possible function of envelope antigen is to be investigated. Crude membranes are being prepared from these cells for immunization and solubilization.

A micro type mixed lymphocyte-tumor cell assay employing thymidine incorporation has been developed in collaboration with Litton Bionetics. This assay has been shown to be specific in causing stimulation of sensitized lymphocytes by tumor cells of the SV40-induced sarcoma mKSA. Soluble antigen (SA) can be substituted for tumor cells and as little as 0.1 μg or optimally 1 μg(SA) induced significant lymphocyte stimulation. This assay is one method now being used to monitor further fractionation and purification of this TSTA.

It was reported previously that we were able to clone neoplastic cells from an MSV(MLV)-induced hemangiosarcoma (XM-1). These clones revealed a heterogeneity as regards their virus production (MLV), presence of defective MSV, transplantability and presence of TSTA. We have continued studies with one of these clones, H-11, adapted to tissue culture growth. This clone continues to show no evidence of infectious virus as determined by sensitive plaque - or focus-forming assays and by the buoyant density gradient assay nor is gs antigen detectable. MSV is not rescuable yet this clone retains a moderately strong TSTA that is MSV(MLV) specific. We are presently further characterizing this TSTA. These results refute the contention of Stephenson & Aaronson and of G. Klein that infectious virus of virion antigens are the effective TSTA in RNA virus-induced neoplasms.

TSTA of plasma cell (PC) tumors have been studied in BALB/c mice. One group of PC, induced by mineral oil was shown to have either individual or in some cases, cross-reacting TSTA. A second group of PC induced by Abelson virus (A-MLV) were shown to have only cross-reacting antigens within the group but not with the TSTA of mineral oil-induced PC. These A-MLV induced PC were producers of C-type virus - thus virion structural components probably are the effective antigens. Since viral structural components (P30, P15, GP 69/71) and antisera to these are now available, we are in a position to distinguish between the role of structural virion components and virus-specified new cellular antigens in tumor rejection, in in vitro cytotoxicity and the relationship between in vivo and in vitro events.

We are initiating work of attempting to solubilize and purify TSTA from methylcholanthrene (MCA) tumors of mice. A previous attempt, employing plasma cell tumors with strong TSTA (e.g. Adj PC5) showed these membrane bound antigens to be excessively labile. Two groups of investigators (Baldwin in England and Rapp & colleagues at NCI) have studied soluble materials which to date at least show little in vivo activity (that is, tumor rejection). Meth-A and MCA induced sarcoma has been adapted to in vitro growth as has MCA-10 and MCA-18; the latter, MCA-18 is known to be free of virion antigens (C type) whereas MCA-10 is infected. In this system a possible role of passenger viruses in providing positivity in in vitro assays will be studied.

Dr. Barry Fernbach (Research Associate) in conjunction with Dr. Guy Bonnard (LID) has developed a new simplified microtechnique for the in vitro generation of cell-mediated (CM) immunity. This should be of advantage where cells, sera or inhibiting substances are in short supply. This material is presently being used to investigate the role of γ-globulin in CM responses and suppressed and "suppressor" cells in the immune response.

^{3}H-thymidine incorporation (TI) has come to be accepted as the sine qua non of lymphocyte proliferation in the mixed lymphocyte reaction (MLR). There is increasing evidence however that this may not be true. Counting the number of "blast" cells is a more basic but time consuming and subjective measurement. It is now possible to follow "MLR" using a semi-automated cell counting technique performed on a "Cytograf." This technique is as sensitive as TI and not subject to the artifacts of TI. We are applying this technique to several areas of study where suppression of TI may or may not be a true reflection of cellular proliferation.

Significance for Cancer Research:

Characterization of tumor antigens of the rejection type is a necessary prerequisite for understanding the mechanisms of immune surveillance, tumor inhibition and facilitation. In addition the role of these membrane bound components in the actual process of carcinogenesis may be studied.

As a basis for any study of membrane bound antigens it is necessary to study the nature of histocompatibility antigens and their relationship to tumor antigens. Thus our emphasis is on parallel studies of H-2 antigens in order to provide a basis for understanding tumor antigens.

Objective 3; Approaches 4,5.

Keyword Descriptors:

VSTA, virus specific tumor antigens; TSTA, tumor specific transplantation antigens, virion antigens, SV40 virus, RNA oncogenic viruses, H-2 (histocompatibility) antigens, β-2 microglobulin, congenic mice, RLV, Rauscher leukemogenic virus, SA (soluble antigen), chromatography.

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Project No. Z01 CBD 03222-07 LCBGY
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic and biochemical mechanisms controlling protein synthesis in mouse plasma cell tumors and other well-differentiated neoplastic cells

Previous Serial Number: NCI-3222

Principal Investigator: J. Frederic Mushinski, M.D.

Other Investigators:
Dr. Michael Potter (Z01 CBD 05596-07 LCBGY)
Dr. Hayden G. Coon, (Z01 CBD 05552-07 LCBGY)
Dr. E. Premkumar Reddy, (Z01 CBD 03222-07 LCBGY)
Dr. I. Bernard Weinstein, Columbia University
Dr. Dezider Grunberger, Columbia University
Dr. Raphael Salomon, Weizman Institute of Science

Cooperating Units:
Columbia University
Weizmann Institute of Science, Rehovot, Israel

Man Years:
Total - 1.83
Professional - 1.00
Other - 0.83

Project Description:

Objectives: To study mammalian control mechanisms in transcription, translation and protein assembly using rodent tumors *in vivo* and *in vitro* as models of human neoplastic and normal cells.

Methods Employed:

A) Transfer RNA from mouse myelomas and rat hepatomas

The striking variations seen in isoaccepting tRNAs when tumors are compared with other tumors or with normal tissues are being studied using reversed phase, high pressure liquid chromatography and polynucleotide directed ribosome binding.

B) IgA half molecule mouse myeloma proteins

In order to understand the genetic and biochemical events responsible for the synthesis and secretion of IgA half molecules (one light chain disulfide bonded to one unusually short heavy chain) we are mobilizing several disciplines and approaches:

1. Developing homologous and heterologous antiserums to this class of IgA.
2. Structural studies on the seven known examples of this unusual Ig.
3. Detecting new examples of this type of tumor as more plasmacytomas are induced or by recognizing mutant clones in cultured IgA - producing plasmacytomas.
4. Isolation of messenger RNA to see if the short heavy chains of IgA half molecules have smaller mRNAs than other IgAs.
5. tRNA comparisons by reversed phase chromatography and cell-free protein synthesis.

Major Findings:

A. Phenylalanyl-tRNA from rat liver and rat hepatomas

We have described the first structural difference between a tRNA found only in tumors and not in normal tissue. A hepatoma specific Phe-tRNA has been found eluting earlier than normal liver Phe-tRNA when reversed phase column chromatography (RPC-5) was used to study Phe-tRNA isolated from three Morris rat hepatomas, 7777, 5123D, and 7800. We have shown that this tumor specific Phe-tRNA$_1$ differs from the single species of normal Phe-tRNA (which is also present in hepatomas as Phe-tRNA$_2$) by lacking a complete Y base (the very highly modified, hydrophobic and fluorescent base characteristically found next to the anticodon of all eukaryotic tRNAs^{Phe}). This was shown by demonstrating less hydrophobic attachment to RPC-5, no sensitivity to dilute acid which normally removes the Y base when it is present, no binding to an antibody specifically reacting to the Y base, and no fluorescence at 435 mμ. This molecule is not just a degradation product of Phe-tRNA$_2$ that lost the Y base during isolation, because Phe-tRNA$_{-Y}$ elutes even earlier than Phe-tRNA$_1$ on RPC-5 chromatography, reacts with MBTH and other aldehyde reagents which Phe-tRNA$_1$ does not and does not bind to Poly(U) treated ribosomes to which Phe-tRNA$_1$ does bind.

Two other features of Phe-tRNA are still being studied. We have detected in rat liver and hepatomas a phenylalanine-tRNA synthetase that will not aminoacylate Phe to tRNA^{Phe} if the Y base has been removed artificially but will aminoacylate Phe to tRNA$_1^{Phe}$ and tRNA$_2^{Phe}$. Previously this type of specificity was thought to be found only in prokaryotic Phe-tRNA synthetase. Additionally, a column of Sephadex G-100 has been found that removes Y base from a percentage of normal liver

$tRNA^{Phe}$. It is being studied for the presence of some condition, contaminating organisms or enzyme that hydrolyzes the Y base off during chromatography.

Although Phe-$tRNA_1$ may be due to the transcription of a new tRNA gene in hepatomas, it is not due to the reactivation of a fetal gene as it has been reported. We have isolated tRNA from adult, newborn, and fetal rat livers as well as mid term fetal rat embryos and can detect no evidence of any $tRNA^{Phe}$ resembling Phe-$tRNA_1$, the tumor specific molecule.

B. IgA half molecules

Preliminary studies on the molecular weight of the entire half molecule as well as separated light and heavy chains from all the examples of this class of IgA indicate that they are all very close to that carefully determined for two prototypes of this type molecule. Determinations of the carboxyl terminal amino acid residues are not yet unambiguous but they suggest that all such half molecule heavy chains end with the same amino acids. These findings are consistent with this type of half molecule IgA being a separate subclass of IgA in the mouse.

Significance to Biomedical Research and the Program of the National Cancer Institute:

A. The finding of a structural difference in a tumor specific tRNA is of considerable significance in our search for biochemical mechanisms responsible for neoplastic transformation. The location of this structural alteration immediately adjacent to the anticodon suggests that it may disturb or alter the fidelity of translation of intracellular messenger RNA, with many potential sequelae in cellular metabolism and growth. Although we have shown that this tumor specific Phe-tRNA is not normally found in fetal cells, it remains of paramount importance to determine whether this represents the transcription of a new gene or an incomplete post-transcriptional modification of this particular tRNA molecule. Additional structural and biological studies should indicate whether or not this tRNA could be the result of a mutational event at the DNA level.

B. We still cannot be certain whether the shorter heavy chains of the IgA half molecules are the result of mutational acts or the expression of a normal but so far undetected IgA subclass. Both mechanisms have great significance in studying the mutational changes possible for somatic structural genes and the genetic arrangement and sequential expression of immunoglobulin genes in normal cells and in plasmacytomas. The recent finding of a human myeloma protein with an unusually short IgA heavy chain broadens the interest in understanding the mechanisms involved in producing short IgA heavy chains in the mouse.

Proposed Course:

A. We are preparing large quantities of the two Phe-tRNAs from hepatoma 7777 in order to do more detailed structural studies and determine if the Y base is the only difference in their structures. Coding studies to determine the effect this structural change has upon fidelity of translation, and DNA hybridization to look for a separate tRNA gene for the tumor specific tRNA in normal and tumor DNA are planned.

A carefully planned series of comparisons of tRNAs from mouse plasmacytomas with secretory proteins of known structure should let us understand several aspects of the control of the expression of tRNA genes whether it is determined by codon content of intracellular messenger, whether it is fixed at the time of neoplastic transformation, and whether it can be varied by treatment with mutagens.

B. Continued structural studies to determine the nature of the defect in the short IgA heavy chains and, hopefully, to learn the genetic mechanisms responsible for generating such molecules are planned. Development of a sensitive immunoassay such as radioimmunoassay or hemagglutination inhibition will be developed to search for this type of IgA in normal mouse serum and body fluids.

Keyword Descriptors:

Transfer RNA, Immunoglobulin A, Rat Hepatoma, Mouse Plasmacytoma, Multiple Myeloma.

Relationship to National Cancer Plan:

Objective - 3

Approach - 1

Publications:

Mushinski, J. F.: Mouse IgA Myeloma Proteins with Alpha Chain Deletions: A Comparison with Human Alpha Chains with Delections. In Mestecky J. and Lawson, A. R. (Eds.) The Immunoglobulin A System. New York, Plenum Publishing Corp., 1974, pp. 223-227.

Grunberger, D., Weinstein, I. B. and Mushinski, J. F.: Deficiency of the Y base in a hepatoma phenylalanine tRNA. Nature 253: 66-67, 1975.

Project No. Z01 CBD 03229-07 LCBGY
1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: I. Chemical structure of immunoglobulin molecules and allotypes
II. Purification and biochemical characterization of soluble murine H-2 antigens, human HL-A antigens, and tumor specific antigens

Previous Serial Number: NCI-3229

Principal Investigator: Dr. Ettore Appella

Other Investigators:
Dr. Ole Henriksen (Z01 CBD 03229-07 LCBGY)
Dr. Michael Rogers (Z01 CBD 03229-07 LCBGY)
Dr. R. Mage (I:LI)
Dr. John Inman (I:LI)
Dr. N. Tanigaki (Roswell Park Memorial Inst., Buffalo, N. Y.)
Dr. O. Roholt (Roswell Park Memorial Inst., Buffalo, N. Y.)
Dr. D. Pressman (Roswell Park Memorial Inst., Buffalo, N. Y.)

Cooperating Units: Roswell Park Memorial Inst., Buffalo, N. Y.

Man Years:
Total - 2.5
Professional - 1.0
Other - 1.5

Project Description:

A) Mouse Immunoglobulins

Mouse myeloma proteins with antigen binding activity toward a variety of antigens have been described. We have been interested in MOPC-511, an IgA immunoglobulin with phosphorylcholine binding activity. The complete amino acid sequence of the light chain and the near complete sequence of the heavy chain have been obtained. A relevant finding in this study is the close similarity of the V_H amino acid sequence of MOPC-511 to the V_H of Mc603, another IgA with the same specificity. Five variable positions have been found in the second and third hypervariable regions; in addition two other positions outside the hypervariable regions have changed. These data seem to suggest that at least two germ line genes are involved in the coding for the heavy chains with phosphorylcholine specificity. For the antigen binding specificity, the heavy chain seems to play a dominant role. The three residues in contact with the phosphate groups of the hapten, Tyr-33, Arg-52 and Lys-54 remain invariant. The choline moiety is involved in a charge interaction with Asp-99 of the heavy chain as well as with Glu-90 of the light chain. The predominant interaction of the hapten with the heavy chain may explain the high degree of conservation of the amino acid sequence of the two heavy chains. The amino acid sequence of the light chains from two other phosphorylcholine proteins differ to a greater extent than any of the heavy chains so far compared.

HISTOCOMPATIBILITY ANTIGENS

Recent reports have demonstrated that purified papain solubilized HL-A antigen molecules consist of two components of dissimilar size bound non-covalently. The smaller component of 11,000 dalton has been shown to be the same as human β2-microglobulin; the large component of 34,000 dalton contains the alloantigenic determinants. The large component can be prepared in the native state by dissociation with anti-human β2-microglobulin or by dissociation at acid pH which results in a loss of alloantigenic activity. Purified preparations containing predominantly HL-A2 or HL-A7 alloantigenic activity were obtained from RPMI 1788 human cell line. A comparison of their physical-chemical properties revealed that these two fragments have the same molecular weight. However, these analyses demonstrated differences in charge between the two types of molecules. Electrofocusing of the HL-A2 molecules on urea-acrylamide gel showed multiple bands having pI values higher than those from HL-A7. Comparison of total amino acid composition showed small but significant differences between these two preparations. Attempts have been made to determine the number of half-cystine residues. By full reduction and alkylation with iodoacetamide four carboxymethyl-cysteines per mole of large component were found. However, fingerprinting carried out on the tryptic peptides revealed five to six radioactive peptides. The exact number of half-cystine remains at the moment uncertain. An accurate determination is being carried out, in view of the possible homology with the constant region of immunoglobulin heavy chains. It has been suggested that the immune system may have arisen in evolution from genes specifying the histocompatibility antigens or portions of these antigens; the structure of the large component should be very enlightening. Recently we have obtained evidence that the H-2 antigens of the mouse also contain two fragments, of about 37,000 and 11,000 dalton. Highly purified papain solubilized antigens can be dissociated into two fragments by exposure to acid pH. By the use of monospecific antisera it was shown that the large component carried H-2 allospecificities and that the H-2 specificities coded by K and D regions of H-2 locus are carried by different molecules. The small component carried binding activity only with a rabbit antiserum against a β2-microglobulin homologue in mice. The 11,000 dalton fragment could also be extracted by 3M NaSCN and purified by successive chromatography and electrophoresis to complete homogeneity. Total amino acid composition has revealed homology with the rat and human 11,000 fragment or β2-microglobulin. Antigenically it cross reacts with rabbit antibodies directed against the Rat Ag-B or human HL-A 11,000 fragment.

Further studies of the biological effects of solubilized $H-2^a$ antigens have been reported. B10.D2 neonatal mice have been injected daily through a period of 4-5 weeks. Twenty-eight preparations revealed activity in inducing antibody tolerance; cell mediate activity was retained as revealed by skin graft rejection. At the present time the mechanism involved in this split tolerance is unknown; if defined, it could have important applications in attempts to manipulate the immunosystem.

TUMOR SPECIFIC TRANSPLANTATION ANTIGENS

Tumor specific transplantation antigens (TSTA) have been studied in two systems, a BALB/c line transformed by SV40 (mKSA) and a C57B/6 line transformed by Rauscher virus (RBL-5). The method used for solubilization of H-2 antigens, limited papain digestion, has been adapted to these tumors in order to obtain immunogenic TSTAs. The immunogenic activity was found to be confined to an included peak on a Sephadex G150 chromatography which in each instance was positive for alloantigenic activity. Specific tumor rejection was achieved in syngeneic mice; in addition specific adoptive tumor neutralization (WINN test) was demonstrated. Cytotoxic antibodies were induced by crude membrane preparations of RBL-5. This humoral response of RBL-5 was further characterized by the microcomplement fixation test. An interesting result was the finding that the syngeneic anti-crude membrane contained an antibody which appeared to react with a non-virion antigen; however, since our best preparation contained virion antigens, the possibility still remains that cytotoxic antibodies directed against virion components on the cell surface play a role. Common antigens arising from activation of endogenous viruses have also not been ruled out. In the mKSA system, by injection of 10^6 viable cells, a subtumorogenic dose of cells, cytotoxic antibodies were not induced. No reactivity was also demonstrated in a ^{51}chromium-release cytotoxicity assay (CRA) at any time following immunization. Lymphocyte stimulation using the mixed lymphocyte tumor cell assay (MLTC) has been performed recently. Activity was demonstrated when immune spleen cells were mixed with the mitomycin-C treated tumor cells or when solubilized antigens were used. This activity parallels the cellular immune reactivity against TSTA and this is possibly the only detectable in vitro activity for future use to characterize this antigen.

Keyword Descriptors:

Mouse: immunoglobulin histocompatibility antigen, mouse and human tumor-specific transplantation antigens; Human: histocompatibility antigens, mouse and human protein chemistry, amino acid sequence.

Publications:

Robinson, E. A., Smith, D. F. and Appella, E.: Chemical characterization of a mouse immunoglobin A heavy chain with a 100-residue deletion. J. Biol. Chem. 249: 6605-6610, 1974.

Law, L. W., Appella, E., Strober, S., Wright, P. W. and Fischetti, T.: Soluble transplantation antigens. Transplantation 18: 487-495, 1974.

Chang, K. S. S., Law, L. W. and Appella, E.: Distinction between tumor specific transplantation antigen and virion antigens in solubilized products from membranes of virus-induced leukemic cells. Int. J. of Cancer 15: 483-493, 1975.

Appella, E., Henriksen, O., Natori, T., Tanigaki, N., Law, L. W. and Pressman, D.: Basic structure of mouse histocompatibility antigens. Trans. Proc. (in press).

Law, L. W. and Appella, E.: Studies of soluble transplantation and tumor antigens. In Becker, F.F. (ed.): Cancer: A Comprehensive Treatise. New York, N. Y., Plenum Press, Vol. II, (in press).

Project No. Z01 CBD 04833-07 LCBGY
1. Laboratory of Cell Biology
2. Ultrastructural Biol. Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electron microscope, biological and biochemical studies of normal, malignant and virus infected cells and cell fractions.

Previous Serial Number: NCI-4833

Principal Investigators:	Dr. Nelson A. Wivel Dr. Stringner Sue Yang Dr. Harry L. Malech
Other Investigators:	Dr. G. Smith (NCI, Laboratory of Biology) Dr. P. G. Calarco (Univ. of Calif.)
Man Years:	Total - 4.00 Professional - 3.00 Other - 1.00

Project Description:

Objectives: The Ultrastructural Biology Section is concerned with the study of various factors which influence cell differentiation and malignant transformation. Particular emphasis is given to systems in which viruses represent the transforming agent. The projects undertaken require the correlation of data from various disciplines such as electron microscopy, virology, biochemistry, and immunology.

Methods Employed: The principal methods involve the visualization of appropriate selected biological materials in the electron microscope, the use of tissue culture for propagation of viruses, the use of experimental animals for propagation of tumors, the use of differential and density gradient centrifugation to isolate cell and virus fractions, the use of radioisotopes to label viral nucleic acids, and the detection of viral antigens by a variety of immunological techniques.

Major Findings:

1. Intracisternal A particles. Using high resolution electron microscopy and a combination of preparative techniques, it has been possible to gain important new information about the structure of intracisternal A particles (IAP). Up to this point all of the studies of IAP have involved either thin sectioning or negative staining. The results reflect essentially what is seen in a one-dimensional plane. Through the use of critical point drying plus platinum shadowing it has been possible to view unsectioned IAP as "whole mount" preparations with three dimensions. Further, latex spheres were added to purified IAP as an internal marker for measurements and for confirmation of particle configuration. Our data indicate that isolated IAP have an average diameter of 85 nanometers, they are essentially uniform spheres as indicated by the shadow length, and they consist of two concentric shells surrounding a hollow core. It can be demonstrated that numerous single IAP have an apparently intact outer shell. This is an important finding because previous reports have suggested that the maturational defect in IAP is a failure of particle buds to be released from the membranes of endoplasmic reticulum. The fact that we can isolate IAP that do not exhibit defects in the outer shell strongly suggests that a certain proportion of these particles are released from the membrane into the cisternal spaces of the endoplasmic reticulum. Our results are buttressed by the fact that the conditions of isolation preclude any synthesis or repair of defects in the outer shell of the particle.

We have completed a collaborative project with Dr. P. Calarco at the Univ. of Calif. which includes a detailed description of the appearance of IAP during early embryogenesis. In tracing the morphologic sequence of events preceding the development of IAP, it was discovered that crystalloid aggregates contiguous with the endoplasmic reticulum appear first in the fertilized egg. These structures increase in size and number until the morula stage. During the blastocyst stage crystalloid units often form densely staining plaques. As these crystalloids continue to develop, budding and complete IAP begin to appear. By the time the embryo reaches the outgrowth stage these crystalloids and plaques can no longer be detected, although IAP can still be found. Through the use of immuno-electron microscopy, it may be possible to determine whether or not these crystalloids and IAP share common antigens.

2. Intracytoplasmic A Particles. In an ongoing collaboration with Dr. G. H. Smith, we have carried out a comparison of intracisternal and intracytoplasmic A particles. Both types of particles have been found within the cytoplasm of the same tumor cell. There are morphological similarities, but the gel electrophoresis patterns of the structural proteins show distinct differences. We have now demonstrated some immunological cross reaction between the two systems, as indicated

by C-F and immunodiffusion assays. This does not appear to involve major structural proteins and most probably reflects common antigenic determinants among minor proteins. The implications of these findings are the subject of continuing studies.

3. Human mononuclear leukocyte IgG receptors. As a recent project, Dr. Harry Malech has been studying surface receptors on human mononuclear leukocytes (HML). It is known that HML possess IgG surface receptors which may be involved in phagocytosis. Such receptors can be detected by the ability of HML to form rosettes with IgG-coated erythrocytes. During phagocytosis this IgG surface receptor activity disappears. Through the use of a chemotactic factor derived from human complement, it is possible to demonstrate that HML possess a definite polarity in their response to such a stimulus. When HML are exposed to a chemotactic gradient across a millipore filter, these cells line up at the filter surface and long pseudopods are thrust into the filter pores. In this condition the tail of the HML cell farthest from the chemotactic stimulus does not form rosettes with IgG coated erythrocytes. This loss of receptor activity is not due to inhibition by chemotactic factor since exposure of cells to this factor on both sides of the filter chamber does not cause similar impairment of rosette formation. In summary, the results indicated that HML lose their IgG binding receptors at the trailing end of the cell in response to a chemotactic stimulus. The mechanism by which these changes occur remains to be determined. It could reflect either internalization, rearrangement of migration of receptors or a combination of such events.

Relationship to National Cancer Plan:

Project Area - 10

Approach Elements - 2

Approach - 4

Keyword Descriptors:

Oncornavirus, tissue culture, electron microscopy, viral immunology, nucleic acid analysis.

Publications:

Yang, S. S., Calarco, P. G., and Wivel, N. A.: Biochemical properties and replication of murine intracisternal A particles during early embryogenesis. Europ. J. Cancer 11: (in press).

Malech, H. L., Schreiber, A. D., and Root, R. K.: Human mononuclear leukocyte IgG receptors: Response to a chemotactic stimulus. Nature (in press).

Project No. Z01 CBD 05526-07 LCBGY
1. Laboratory of Cell Biology
2. Macromolecular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell surface changes in normal, DNA virus transformed and spontaneously transformed tumorigenic mouse cell lines.

Previous Serial Number: NCI-5526

Principal Investigator: Peter T. Mora, Ph.D.

Other Investigators:
Dr. S. Luborsky (Z01 CBD 05544-07 LCBGY)
Dr. J. Coll (Visiting Fellow)
Dr. C. Chang (Visiting Fellow)
Dr. S. Pancake (Guest Worker)
Mrs. V. McFarland (LCBGY)
Ms. L. Couvillion (LCBGY)
Dr. R. G. Martin (LMB, NIAMDD)
Dr. P. W. Kent, University of Durham
Dr. A. Schultz, Biotech Research Laboratories, Inc.

Cooperating Units:
University of Durham, Glycoprotein Research Unit, Durham, England
Biotech Research Laboratories, Inc., Rockville, Maryland

Man Years:
Total - 6.2
Professional - 3.2
Other - 3

Project Description:

Objectives: To elucidate certain biochemical changes on the cell surface which occur during transformation to malignancy in mouse cells.

Methods Employed: Labelling glycoproteins, proteins and glycolipids, also of nucleic acids with radioactive precursors during cell growth in culture. Cell homogenization and subcellular fractionation techniques such as isolation of cell membranes. Chemical isolation and characterization procedures, including thin-layer chromatography, gel electrophoresis, autoradiography of gel slabs. Analytical and preparative ultracentrifuge studies. Electronmicroscopy. Special enzymology techniques pertinent to intermediary metabolism of carbohydrates. Serum mediated cytotoxicity assays as measured by release of radioactivity from lysed cells. Cell growth dynamics in culture and *in vivo*.

Major Findings:

I. Biochemically-induced modification of the cell surface membrane carbohydrates.

The surfaces of mammalian cells have long been known to contain complex carbohydrate materials that often lack clearly recognizable roles. More recently, it was observed that cultured cells exposed to a variety of viral or environmental agents "transform" and acquire altered *in vitro* and *in vivo* growth properties. Glycoprotein and glycolipid changes in cell-surface membranes appear to accompany certain of such transformations. Whether the structural modifications of these macromolecules are the causes of the new cellular behavior, or are merely secondary consequences of the "transformation", has not yet been established.

Direct modification of cell-surface carbohydrates would be a first step in an attempt to establish any correlation between surface carbohydrates and their biologic function in mediating cell behavior. Furthermore, it may be of use to resolve the question of causal relationship between changes in the biological properties and the carbohydrate components in the cell surface.

Previous studies with Dr. P. W. Kent have shown that up to a final concentration of 0.5mM, 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose, commonly named N-fluoroacetyl-D-glucosamine, is nontoxic to mouse cells in culture, and may affect the synthesis of cell surface sialic acid (N-acetylneuraminic acid), the terminal and negatively charged carbohydrate unit in many glycoproteins and glycolipids.

A systematic study was undertaken with A. Schultz to achieve through internal (metabolic) means modification of the sialic acid biosynthesis by N-fluoro-acetyl-D-glucosamine (Glc NAc F).

Addition of 0.5mM Glc NAc F to extract of mouse cells was studied, by the use of various metabolic intermediates of glycoproteins and glycolipids as tracers. These tracers included 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-mannopyranose and N-acetylneuraminic acid. It was found that Glc NAc F drastically inhibited the accumulation of two nucleotide sugars: uridine 5'(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate and cytidine 5'-(N-acetylneuraminyl monophosphate. These nucleotide sugars are essential metabolic intermediates in the pathway of sialic acid incorporation into glycoproteins and glycolipids.

These studies have shown how a non-toxic metabolic modifier (Glc NAc F), a close analog of the very common carbohydrate precursor of N-acetyl-glucosamine, can establish a new steady state pool of precursors for glycolipid and glycoprotein biosynthesis. Replacement of hydrogen by fluorine results in little steric change, but the vastly different electronegativity has a definite effect on the interaction of a fluorine-containing molecule with the enzymes participating in the above biosynthetic processes. External modification of carbohydrate material with glycosidases were hampered by limited access to substrate, owing to the apparently complex structure of the cell periphery; moreover, glycosidases are often contaminated with extraneous enzymic activities. Furthermore the enzymic modifications are only transitory, as cells repair their surfaces. The metabolic method discovered, and the clarification of the mechanism opens important new ways to study the biologic consequences of alteration of sialic acid residues in the highly hydrophilic glycoproteins on the outermost cell surface, including the role of this residue in cell interaction, neoplasia and tumor rejection.

II. Cell membranes changes in spontaneous and viral transformation.

Recent claims from various laboratories indicate that upon transformation of cells such as hamster cells, in culture with various tumorigenic viruses for example with SV40, there is a decrease in certain high molecular weight cell surface proteins. We have developed cell lines from AL/N mouse, including normal, non-tumorigenic fibroblast lines (N AL/N), spontaneously transformed highly tumorigenic lines (T AL/N), a clonal derivative line (T AL/N Cl 3) and also several carefully matched derivative subclones of this clonal line with or without additional transformation by SV40 (T antigen positive and negative subclones), but with no difference in the growth properties of these cells on solid substratum (no difference in contact inhibition of cell division).

The composition of isolated cell surface membranes from the various lines was compared by different techniques, including labelling of cell membrane proteins (glycoproteins) both from the "inside" metabolically, and also from the "outside" chemically such as reacting the surface protein NH_2 groups on the intact cells with pyridoxal phosphate and NaB^3H_4 (see Project No. Z01 CBD 05544-07 LCBGY). There were no detectable differences in the gel electrophoretic patterns of surface membrane proteins labelled by this last mentioned method, or of glycoproteins labelled by metabolic incorporation of ^3H glucosamine. However, when the cells were labelled metabolically with ^{14}C amino-acid mixture for extended period of logarithmic growth (7 hrs.), the normal cells as contrast to all of the other (spontaneously or virally) transformed cells incorporated substantially more amino acids into (a) high molecular weight protein(s). Careful analysis on slab gel electrophoresis by autoradiography shows that the deficiency in such metabolic incorporation of amino acids into (glyco?) proteins in all transformed cells is indeed reproducible and significant, it is not a consequence of tissue culture artifacts, and affects only selected few (one or two out of about 40) well identifiable (glyco?) protein components (sharp peaks on gel electrophoresis) in the cell surface membrane. The biological significance of this biochemical finding is not clear at the present. One conclusion one can make at this time is that since the surface membranes of SV40 transformed T antigen positive sister subclones showed almost identical protein constitution as compared to the T antigen negative subclones, any SV40 specific surface cell antigen which we detect, such as by the direct cell killing (^{51}Cr release) or indirect inhibition technique employing specific antisera, must be only a very minor constitutional component of the cell surface membrane. This, of course, offers fair warning that in solubilized (glyco)protein components from cells, even from purified cell surface membranes and even after various biochemical purification techniques, one can only expect very minor quantities of SV40 specific antigens to occur in the presence of very large excess of other protein(s).

III. Cell properties after repeated transplantation of spontaneously and of SV40 virus transformed cell lines.

A study was directed to find the changes in cell surface and phenotypic growth properties which result as a consequence of repeated transplantations as tumors of established cell lines in the syngeneic AL/N mouse. Both spontaneously and by SV40 transformed cell lines thus studied. In comparing tissue culture growth properties, it was found that the SV40 transformed cells, including the derivative lines reestablished as cell lines after repeated transplantations, all grew well in suspension in a medium made viscous by methyl cellulose (10-20% plating efficiency). However, in contrast to recent claims on other cells that ability to grow in methyl cellulose medium is a general correlate of tumorigenicity, the "spontaneously" transformed cells and the derivative cells which are all very highly tumorigenic (TD_{50} ∿ 50 cells/mouse) grew only poorly (0.03-1%). There were no differences between the spontaneously and virally transformed cells and all of their derivative cells reestablished after passage as tumor, in the cell growth measurements used in tissue culture by many viral oncologists such as in "saturation density", in rate of growth in 10% or 1% serum, when the cells were grown attached to the substratum. This shows that commonly employed tissue culture growth parameters of cells growing on substratum cannot be used to distinguish reliably between virus transformed cells and spontaneously transformed cells, neither between highly and poorly tumorigenic cells, but the ability to grow in methyl cellulose is a reliable hallmark of the SV40 transformation in the cell system studied. All of the SV40 transformed cells including the derivative cells continued to express T antigen and continued to react in vitro in a cytotoxicity assay with specific antisera, and all had relatively low tumorigenicity (TD_{50} 10^5 - 10^8). This implied that the lower tumorigenicity of the SV40 transformed cells, including the derivative lines obtained after repeated transplantation in the syngeneic mouse, is due to expression of tumor and viral specific transplantation antigen(s). Biological tests support the interpretation that in the AL/N mouse cells the dominant biologic consequence of SV40 virus transformation is in vivo recognition and rejection.

Two methods were used to reestablish cell lines: 1. trypsinization, 2. replating of small minced pieces of the tumors under high (20% serum concentration. In the derivative cell lines from the tumors induced by the spontaneously transformed cells the cells' ability to grow in methocell was reduced by two orders of magnitude, when the lines were reestablished by the second technique as compared to the first. Apparently, while trypsinization dispersed substantially all of the tumor (fibrosarcoma) cells indiscriminately of their surface properties, the few cells which detached from the minced tumor pieces and then re-attached to the substratum were selected cells, which had low adhesiveness to another tumor cell, an ability to survive in concentrated fetal calf serum, a propensity for attachment to the dish which was coated by serum and also a need for attachment to solid substratum to be able to multiply. This might have some pertinence to, or serve as a model to study in vivo metastasis.

IV. Reduction of tumorigenicity of spontaneously transformed AL/N mouse cells when further transformed by SV40.

As outlined above, spontaneously transformed very highly tumorigenic cell line (T AL/N; TD_{50} ∿ 50 cells/mouse) was cloned, and after exposure to SV40 virus several subclones were obtained, some T antigen positive, some T antigen negative. The T antigen negative subclones maintained the high tumorigenicity, but the T antigen positive clones had much reduced tumorigenicity (TD_{50} ∿ 10,000 cells/mouse). The latter cells also grew well in methocell, and had SV40 induced cell surface antigens detectable by absorption of the specific cytotoxic antisera in ^{51}Cr release assay. The obvious implication that the increased immunogenicity is causing the reduced tumorigenicity is being supported by in vivo experiments. Thus, in the AL/N mouse, SV40 virus which is tumorigenic in another species (hamster) not only endows the normal cells with cell surface properties which result in recognition and rejection, but it can overcome the tumorigenic potential of very strongly tumorigenic cells.

The membrane constitution of all the subclones and also derivative cell lines after repeated transplantations is similar, but significant minor differences are being observed. These differences in cell surfaces are being further related to the phenotypic growth properties of these cells, both in vivo and in tissue culture. The above described AL/N mouse cell lines with graded tumorigenicity and rejection are of special value to detect biochemical differences in cell surface membranes, and to systematically relate these to the phenotypic growth properties, and also to the genotypic changes which occur in spontaneous and in SV40 virus induced transformation. In the last context, collaborative studies are in progress (with R. G. Martin, LMB, NIAMDD) using SV40 mutant viruses temperature sensitive to transformation in certain growth properties of cells in tissue culture.

The above research shows for the first time that highly tumorigenic mouse cells transformed spontaneously by an unknown process, can be rendered essentially non-tumorigenic by SV40 viral transformation through expression of strong new cell surface antigens.

Other spontaneously transformed mouse cell lines, their clonal derivatives, and recloned lines after SV40 transformation are under study to define the generality and the obligatoriness of the reversal of cell tumorigenicity by viral transformation.

Significance to Biomedical Research and the Program of the National Cancer Institute:

Studies of cell surface biochemical changes which relate to acquisition of tumorigenic potential or to immunologic recognition and rejection in vivo are of interest, especially when they may lead to understanding of changes in molecular mechanism which appears to be reducible to heritable changes on control processes on the DNA level in the virally induced transformation of cells. This work pertains to the National Cancer Plan Objective 3: Develop the means to prevent transformation of normal cells to cells capable of forming cancers; Approach 2: Delineate the nature and rate of oncogenic cell transformation in carcinogenesis (including aspects of cell culture, viruses), and to Approach 5: Study of cell surface and cell membranes.

Proposed Course of the Project:

There will be an attempt to further clarify the relevance of the various changes in cell membrane biochemistry (glycoproteins, proteins, etc.) to spontaneous and to viral induced transformation of cells. We also plan to relate these changes, and the changes caused by the amino-sugar biosynthesis inhibitor, to phenotypic changes in cell immunogenicity and in tumorigenicity in syngeneic and allogeneic mouse systems. We hope that the information and methodology developed in the mouse system will be of use on human tumor cells.

Keyword Descriptors:

Cell surface membrane, spontaneous transformation, SV40 virus, virus specific antigens, surface antigens, glycoproteins, cell surface proteins, labelling of cell surface, gel electrophoresis, slab gel autoradiography.

Publications:

Pancake, S. J., and Mora, P. T.: Comparison of SV40 induced antigens on the surface of cultivated cells by a cytolytic microassay. Virology 59: 323-327, 1974.

McFarland, V. W., Mora, P. T., Schultz, A., and Pancake, S.: Cell properties after repeated transplantation of spontaneously and of SV40 virus transformed mouse cell lines. 1. Growth in culture. J. Cell. Physiology 85: 101-111, 1975.

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Mora, P. T.: Cell transformation in culture: Pertinence to malignancy: Summary of status and prospects. In Mora, P.T. (ed.) Cell Surfaces and Malignancy. Fogarty International Center Proceedings No. 28, Washington, D. C. U.S. Government Printing Office, 1975. In press.

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Project No. Z01 CBD 05543-07 LCBGY
1. Laboratory of Cell Biology
2. Macromolecular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The relationship of intramolecular interactions to biological function.

Previous Serial Number: NCI-5543

Principal Investigator: Sidney Shifrin, Ph.D.

Other Investigators: Dr. A. S. Levine, NIAMDD
Dr. G. Ashwell, NIAMDD

Man Years: Total - 2
Professional - 1
Other - 1

Project Description:

Objectives: The purpose of this project is to determine the relationship between the quaternary structure of proteins and glycoproteins and their biological function. These studies were carried out with L-asparaginase from *Escherichia coli*, sickle cell hemoglobin, and a glycoprotein isolated from the membrane of liver parenchymal cells.

Methods Employed: Ultraviolet absorption spectroscopy, difference spectroscopy, fluorescence spectroscopy, circular dichroism, sedimentation velocity ultracentrifugation, polyacrylamide disc gel electrophoresis, enzymatic and chemical identification of carbohydrates in glycopeptides.

Major Findings:

A. Denatured subunits of L-asparaginase refold to their native conformation within 30 seconds after removal from the denaturing agent. Reassembly of the folded subunits into the tetrameric aggregate is a very slow process. The rate of reassembly is accelerated by the addition of the natural substrate, L-asparagine, or its isomer, D-asparagine, but is not accelerated by L- or D- glutamine, an amino acid that is slowly hydrolyzed by L-asparaginase.

The quaternary structure of L-asparaginase is stabilized by polyhydric alcohols. The lower polyalcohols such as ethylene glycol and glycerol at low concentrations accelerate the rate of dissociation of subunits in urea. Polyalcohols including erythritol, arabitol, sorbitol and inositol stabilize the quaternary structure of the enzyme.

B. Aggregation of sickle cell hemoglobin is inhibited by the presence of methanol, ethanol and isopropyl alcohol. Aggregation is also prevented by the addition of ethylene glycol, glycerol and erythritol to the association medium. However, aggregation is enhanced by the presence of arabitol, sorbitol, mannitol and inositol.

C. The terminal sialic acid residue of the glycopeptide moiety of binding protein isolated from membranes of liver cells is essential for the binding of asialoglycoproteins. The sequence of sugar residues in this glycopeptide has so far been found to be: sialic acid - galactose - N-acetylglucosamine - α-mannose - . The sequence of sugars closer to the amino acid residues is currently being examined.

D. Succinylation of the binding protein causes the high molecular weight aggregate to dissociate into smaller subunits. While the native material is a heterogeneous aggregate with no distinct sedimentation coefficient, the succinylated binding protein shows two boundaries in the ultracentrifuge. The slower moving material has an observed sedimentation coefficient of 2.2S and the heavier material has a sedimentation coefficient of 7.7S. In the presence of 1μM Ca^{+2} the more rapidly sedimenting material aggregates to a species having a sedimentation coefficient of 14.1S while the value of the slower component increases to 2.7S.

Significance to Biomedical Research and the Program of the National Cancer Institute:

An elucidation of the structural features of proteins and glycoproteins should provide a basis for understanding how cells interact or fail to interact with one another. In the case of sickle cell hemoglobin there is a correlation between the extent of aggregation and the severity of the disease of sickle cell anemia. Also preliminary studies of hepatomas indicated the absence of the binding protein. This project pertains to the National Cancer Plan Objective 6 - Approach 2.

Proposed Course of the Project:

Further examination of the binding protein obtained from liver membranes will be made as to the structure of the protein as well as the glycopeptide moiety in an effort to establish the features which allow this protein to bind asialoglycoproteins.

An effort will also be made to react L-asparaginase with a glycopeptide with a known sequence of carbohydrate units and having a terminal sialic acid. The influence of the glycopeptide on the half time of circulation and on the enzyme activity will be determined.

Keyword Descriptors:

Enzymes, protein-protein interaction, intramolecular interactions, biological function and quaternary structure.

Publications:

Shifrin, S., and Parrott, C. L.: In vitro assembly of L-asparaginase subunits. J. Biol. Chem. 249: 4175-4180, 1974.

Shifrin, S., and Parrott, C. L.: Influence of glycerol and other polyhydric alcohols on the quaternary structure of an oligomeric protein. Arch. Biochem. Biophys. 166: 426-432, 1975.

Project No. Z01 CBD 05544-07 LCBGY
1. Laboratory of Cell Biology
2. Macromolecular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and comparison of cell membranes of normal and transformed cells.

Previous Serial Number: NCI-5544

Principal Investigator: Samuel W. Luborsky, Ph.D.

Other Investigators: Dr. Peter T. Mora (Z01 CBD 05526-07 LCBGY)
Dr. Samuel Pancake (Z01 CBD 05544-07 LCBGY)

Man Years: Total - 1.5
Professional - 1.0
Others - 0.5

Project Description:

Objectives: As part of a study of surface changes in transformed cells we wished to develop methods to isolate cell surface membranes to compare those isolated from cloned transformed mouse embryo cells with those from untransformed, cloned, sister cell lines.

Methods Employed: Tissue culture cell cultivation, infection with SV40 virus, cell cloning procedures, standard biochemical techniques, polyacrylamide gel electrophoresis. Protein was determined by the Lowry procedure.

Major Findings: Cell membranes were isolated using the technique previously described. This involves breaking the cells in a Dounce homogenizer after a period of swelling in hypotonic medium, and purifying the membrane fraction by twice collecting it following centrifugation from the interface between 30 and 45% (w/w) sucrose solutions. The cells were labelled chemically by reaction of pyridoxal phosphate and NaB^3H_4 with their surfaces, or metabolically by exposure for 24 hours to either ^3H-man NH_2 ($2\mu c/ml$) or ^3H-glcNH_2 ($5\mu c/ml$), followed by a "chase" period of 24 hours in non-radioactive medium.

The cell lines tested are continuous culture lines derived from AL/N mouse fibroblasts. Some of these lines have become spontaneously transformed, others have been transformed by infection with SV40 virus. Some cells were cloned both before and after treatment with SV40 virus, and a series of SV40 T antigen positive and negative cloned lines were developed.

Cells were labelled while in their logarithmic growth phase. We obtained substantially similar distributions and recoveries of label in the subcellular fractions in all preparations, with over 80% of the label of the purified membrane fraction found incorporated in high molecular weight (non-dialyzable) form. This was true for T antigen positive (subclones 1,2,3,11) and negative (subclones 5,6,8,12) cell lines, as well as for the normal, the spontaneously transformed, and the SV40 infected uncloned lines (N_L-AL/N, T-AL/N, and SV-AL/N), labelled either with the carbohydrate precursors or with the NaB^3H_4 technique. Upon analysis of the purified, labelled membrane fractions from these cell lines by disc gel electrophoresis, certain features emerged quite reproducibly. The disc gel patterns possessed a large peak at the front (mol. wt. below 17,500), usually a smaller peak in the 50,000 mol. wt. region, a trough near the 150,000 mol. wt. region, and some radioactivity left near the origin. Within this framework, reproducible differences between cell lines, either in number, size, or position of components, cannot be discerned. We conclude that for cells in logarithmic growth, under our conditions there are no differences discernable in the composition of the cell membranes.

Keyword Descriptors: Surface changes, transformed cells, tissue culture, polyacrylamide gel electrophoresis.

Significance for Biomedical Research and the Program of the National Cancer Institute:

Cell growth characteristics and the ability of certain cells to form tumors in animals depend upon a complex set of interactions between the cell and its host, many aspects of which are thought to be affected by the properties of the cell surface, its chemical composition, structure, and antigenic determinants. These properties are only poorly understood. Methods such as those outlined here must be developed for the reproducible isolation of cell membranes for further study. Any differences in the biochemistry of the membranes from normal, "spontaneously" transformed, or virally transformed cells should provide important clues to a better understanding of these different states, and potentially provide means for better immunological control of cancer. This project pertains to the National Cancer Plan Objective 3: Develop the means to prevent transformation of normal cells capable of forming cancer; Approach 2: Delineate the nature and rate of oncogenic cell transformations in carcinogenesis (include aspects of cell culture, viruses); and Approach 5: Study of cell surface and cell membranes.

Proposed Course of the Project: Experiments are under way to test for membrane differences among these cell lines which may depend upon the growth state of the cell, time after plating, or cell density. Different labelling procedures for the cell surface membrane will be compared, both before and after treatment of the labelled cells with trypsin or neuraminidase, to further test the specificity of these labelling reactions. Other normal, spontaneously transformed, and SV40 infected T antigen positive and negative sister clone lines will be examined. The nature of membrane compositional differences will be analyzed by polyacrylamide disc gel electrophoresis including radioautography of separated labelled components. Our aim will be to seek a consistent common protein and/or glycoprotein change among many cloned cell lines having a common history and differing only in their possession of the SV40 T antigen, and to seek to correlate any such differences with other biological properties of the cells, such as their growth state, their antigenicity, and their tumorigenicity.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 05550-07 LCBGY
1. Laboratory of Cell Biology
2. Viral Oncogenesis Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on oncogenic viruses

Previous Serial Number: NCI-5550

Principal Investigator: Kenneth S. S. Chang, M.D., Sc.D.

Other Investigators: Dr. L. W. Law (Z01 CBD 03220-07 LCBGY)
Dr. T. Aoki (C:VLL)
Dr. E. Appella (Z01 CBD 03229-07 LCBGY)
Dr. M. C. Y. Chen (I:LBV)
Dr. N. P. Salzman (I:LBV)
Dr. Wu-Tse Liu (Visiting Fellow)

Cooperating Units: Immunology Section, Viral Leukemia and Lymphoma Branch, NCI and Laboratory of Biology of Viruses, NIAID.

Man Years:
Total - 3.0
Professional - 1.5
Other - 1.5

Project Description:

I. Virological and immunological studies of reticulum cell neoplasms in mice. D1-murine leukemia virus (MuLV), an N-tropic type C virus, was isolated from a spontaneous reticulum cell neoplasm, type B (RCN-B), of an SJL/J mouse. Its distinctive type-specific envelope antigen and ability to induce a lymphocytic leukemia in SJL/J mice injected as newborns have been described. However, no RCN-B neoplasms have been induced to date.

BALB/c mice were used in the present study to determine the pathogenic potential of D1-MuLV. When injected into BALB/c mice 1 day after neonatal thymectomy, 30% of the inoculated mice developed RCN-B in 5 months, whereas none of the uninoculated controls did. From the spleen and lymph node extracts of all RCN-B-bearing mice tested, B-tropic type C viruses (designated E1-MuLV) were isolated in high titers (10^5 - 10^6 XC-plaque forming units/ml). Only low titers (10^1 - 10^2 XC-plaque forming units/ml) of N- or B-tropic viruses were isolated from those thymectomized mice inoculated but non-tumorous, whereas only N-tropic viruses were detected in the uninoculated thymectomized mice. No virus was isolated from the nonthymectomized, untreated controls. Antigenically, the viral envelope antigen (VEA) of E1-MuLV was distinct from those of D1-MuLV, x-VEA, or Gross-VEA, but some cross-reaction with AKR-MuLV-VEA was observed. For the following reasons, it was suggested that D1-MuLV was specifically related to RCN-B oncogenesis in thymectomized BALB/c mice, and that E1-MuLV was selectively activated in RCN-B cells. Injection of other viruses such as Gross- or AKR-MuLV did not result in induction of RCN-B in thymectomized BALB/c mice. If the RCN-B was induced by E1-MuLV, it would be necessary for the E1-MuLV to be activated by D1-MuLV and replicated to a high titer before RCN-B would be induced. In subsequent experiments in which the spleens and lymph nodes of a group of BALB/c mice (neonatally thymectomized and inoculated with D1-MuLV) were tested, we could not detect infectious B-tropic viruses up to the age of 100 days. This finding tends to exclude that E1-MuLV was responsible for RCN-B oncogenesis. A poorly differentiated mammary adenocarcinoma of an uninoculated control (thymectomized) mouse did not contain infectious type C viruses, which suggests that the activation of B-tropic virus in those RCN-B is not merely a consequence of neoplastic change per se. The importance of this observation lies in the fact that a covert endogenous virus may be activated in the neoplastic cells by inoculation of another infectious type C virus.

Further studies are in progress in regard to the role of xenotropic viruses that may be present in these systems. Immunologic reactivities of these RCN-B as target or attacker cells in cell-mediated cytotoxicity test are being investigated.

Significance for Biomedical Research and the Program of the National Cancer Institute:

RCN-B in mice may be a model for study of human Hodgkin's disease, for its pathogenesis, viral genome expression and immunologic reactivities. Elucidation of the role of endogenous type C viruses in oncogenesis in SJL/J and BALB/c mice, and activation of covert endogenous type C virus genome by an infectious type C virus exogenously introduced into the host may throw light on their oncogenic mechanisms and may lead to prevention of neoplastic transformation.

Relationship to National Cancer Plan:

Objective - 3

Approach - 2

II. Immunologic and virologic differentiation of tumor antigen from virion antigens in an RNA virus induced leukemia, and studies on a nonproducer tumor.

Previous studies of solubilized membrane antigen from RBL-5, a Rauscher-MuLV induced leukemia, strongly indicated the distinctiveness of tumor specific transplantation antigen (TSTA) from type C virion antigen by complement fixation and humoral cytotoxicity-inhibition assays using antiviral and syngeneic anti-crude membrane (CM) serum.

Further studies on anti-CM serum samples collected at various intervals during a course of repeated immunization revealed that some serum samples with moderate cytotoxic titers were devoid of antiviral activity by complement fixation and neutralization tests, while higher cytotoxic activities were accompanied by the rise in the titers of antiviral antibodies.

It was found that RBL-3, another tumor induced by Rauscher-MuLV in the same manner as RBL-5, and carried in *in vitro* culture, was a nonproducer, in that no infectious Rauscher-MuLV was detectable. This cell line is currently being studied intensively. The cultured cells were immunogenic in C57BL/6 (B6) mice against challenge with FBL-3, a Friend-MuLV-induced tumor, and immunization of B6 mice with the latter induced an immunity against challenge with RBL-3 tumor (adapted back to *in vivo* ascites growth from the culture). Furthermore, immunization of B6 mice with MSV(RLV) induced resistance against RBL-3 challenge. These facts establish that RBL-3 cells have FMR group cell surface antigen (TSTA type). By complement fixation test this RBL-3 culture was shown to possess *gs* antigen. The presence of envelope antigen is to be investigated. Crude membranes are being prepared from these cells for immunization and solubilization.

After finding a small number of type C particles in the RBL-3 culture, and after an intensive search for susceptible indicator cells, a xenotropic virus was recovered from the RBL-3 cells, and its virologic and immunologic properties are being investigated.

Cell-mediated ^{51}Cr-release cytotoxicity as well as complement-dependent humoral antibody cytotoxicity assays are being conducted to assess the abilities of various subviral components of Rauscher-MuLV to inhibit these reactions.

Significance for Biomedical Research and the Program of the National Cancer Institute:

The evidences for the ubiquitous presence of type C virus genome in mammalian cells are accumulating. The functions of viral genome to induce cell transformation or to produce TSTA with or without concomitant production of virion antigens may be explored for future exploitation for prevention of cancer. Solubilization and purification of nonvirion TSTA from virus-producer and nonproducer cells would provide an understanding of the factors of immunological surveillance, and a possibility of immunotherapy and prevention of neoplasia.

III. Studies on Polyoma Virus DNA.

In the course of study for type C viruses in various murine tumors, a small-plaque polyoma virus, MPC-1, was isolated from a plasmacytoma, 104E, that had been passaged in BALB/c mice. The virus was plaque-purified 3 times in NIH Swiss embryo cells. In cooperation with Drs. Chen and Salzman, the following experiments for mapping the circular DNA genome of MPC-1 virus were conducted.

As a first step toward localization of functional loci of polyoma virus in its genome, the DNA of this virus was cleaved with a restriction enzyme purified from *Haemophilus influenzae* (*Hin* d) and the molecular weights of the limit products were analyzed by electrophoresis and electron microscopy. The 4 fragments (A,B,C, and D, with Molecular weight $(x10^{-5})$ 18.2, 7.9, 2.9 and 2.9, respectively) produced by this enzyme have been ordered by analysis of partial digest products. A physical map of the polyoma virus genome was then constructed.

Significance for Biomedical Research and the Program of the National Cancer Insitute:

Localization of oncogene and other functional loci in these fragments of polyoma virus DNA would be a major step toward understanding of mechanisms of transformation and other virus induced changes in the infected cells.

Relationship to National Cancer Plan:

Objective - 3

Approach - 2

Keyword Descriptors:

Type C virus activation, reticulum cell neoplasms, nonvirion tumor antigen, polyoma virus DNA.

Publications:

Chang, K. S. S., Aoki, T., and Law, L. W.: Isolation of a B-tropic type C virus from reticulum cell neoplasms induced in BALB/c mice by SJL/J type C virus. *J. Nat. Cancer Inst.*, 54: 83-87, 1975.

Chang, K. S. S., Law, L. W., and Appella, E.: Distinction between tumor-specific transplantation antigen and virion antigens in solubilized products from membranes of virus-induced leukemic cells. *Int. J. Cancer*, 15: 483-493, 1975.

Chen, M. C. Y., Chang, K. S. S., and Salzman, N. P.: Studies of polyoma virus DNA: Cleavage map of the polyoma virus genome. J. Virol., 15: 191-198, 1975.

Herberman, R. B., Aoki, T., Nunn, M., Lavrin, D. H., Soares, N., Gazdar, A., Holden, H., and Chang, K. S. S.: Specificity of ^{51}Cr release cytotoxicity of lymphocytes immune to murine sarcoma virus. J. Nat. Cancer Inst., 53: 1103-1111, 1974.

Law, L. W., Appella, E., Chang, K. S. S., and Henriksen, O.: Soluble tumor rejection antigens from membranes of virally transformed neoplastic cells. Proc. XIth Int. Cancer Congress (Florence, Italy) (in press).

Project No. Z01 CBD 05552-07 LCBGY
1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Production and analysis of hybrid somatic cells: their application to some problems of importance in biology and medicine.

Previous Serial Number: NCI-5552

Principal Investigator: Hayden G. Coon, Ph.D.

Other Investigators:
Dr. G. David Trisler (LCBGY)
Dr. Premkumar Reddy (Visiting Fellow)
Dr. Jay Robbins and Dr. Kenneth Kraemer (C:D)
Dr. H. V. Gelboin and Dr. J. Whitlock (C:CH)
Dr. Saverio Ambesi-Impiombato (C:LPHY)
Dr. Marshall Nirenberg and Dr. Alan Peterkofsky (H:LBG)
Dr. Nelson Wivel (Z01 CBD 04833-07 LCBGY)
Dr. Harry Malech (Z01 CBD 04833-07 LCBGY)
Dr. Michael Potter (Z01 CBD 05596-07 LCBGY)

Man Years:
Total - 2.9
Professional - 1.00
Other - 1.9

Project Description:

1) In collaboration with Dr. David Trisler it has been found using a novel assay for thymidine kinase (TK) developed by him that hybrids between TK^- rodent cells and chicken erythrocytes (TK^+) do not retain the chicken enzyme for long periods of time but rather that the hybrids learn to use a secondary (adult) form of the enzyme which they had all along.

It has been known for some time that vertebrate cells possess at least three electrophoretically separable forms of thymidine kinase, a mitochondrial form whose activity is found only in mitochondria, a form active in S-phase synthesis known as the "embryonic" or "tumor" or "logphase" form of the enzyme and another form found almost exclusively in mitotically quiescent cells known as the adult or lag-phase form of the enzyme. The usual BUdR-resistant mutant which is TK^- and is killed in HAT selective medium Trisler has shown is lacking in the "embryonic" or "S-phase" form of the enzyme. Rodent x chicken hybrids may briefly show the chicken embryonic form but the hybrids soon possess only the stationary phase or adult form of the enzyme appropriate to the rodent species. The rodent cells have been altered by the hybridization so that they can utilize their adult form of TK to accomplish S-phase synthesis in HAT selective medium. Their control circuits have become permanently altered. This half of the HAT selective system is used frequently in hybridization work and these findings represent a major advance in tying up many of the unexplained and "mysterious" results observed in this work of the past decade. We have started to speculate upon the "normal" functions of the "adult" form of the thymidine kinase which is ordinarily inaccessible during the S-phase preceeding mitosis. It was first observed that the TK^- cells could accomplish $TdR-H^3$ incorporation even in HAT medium at rates which were reminiscent of repair synthesis. Subsequent experiments have proven that such cells do use TdR in HAT medium after UV-irradiation. We speculate that the "adult" form of the enzyme is used when repair synthesis is engaged and consequently have begun a search among the five known complementation groups of xeroderma pigmentosum which have previously been studied in this laboratory.

2) In collaboration with Drs. Harry Malech and Nelson Wivel (Z01 CBD 04833-07 LCBGY), we have succeeded in developing a method which permits rapid enrichment of "hybrid" populations for cells which have received either a whole cell or a portion of a cell which has been enucleated using the cytochalazin B technique. Selection is possible when the parent donating the cytoplast has been made Ouabain resistant and the recently fused cytoplast plus Ouabain-sensitive nucleated parent line are treated with a 36-48 hour pulse of Ouabain. Ouabain kills sensitive (wildtype) cells by poisoning their Na,K activated ATP-ase so that concentration of K^+ does not proceed fast enough. In resistant cells Ouabain probably attaches but does not poison the K^+ pump. The mutant is, therefore, dominant (a few Ouabain resistant enzyme sites appear to allow sufficient K^+ concentration for cell survival. In particular, a sensitive cell can be rescued for a short period of time by prior fusion with a resistant cell's membrane and cytoplasm. Luckily, the turnover time of the enzyme in the membrane must be slow enough so that "hybrids" survive 36-48 hours in Ouabain, time enough to kill off all those sensitive cells which did not receive such a cytoplasmic supplement. Of course, since only cytoplasm and not nuclear DNA have been contributed by the Ouabain resistant "cytoplast" the mutant phenotype can not last much beyond the 48 hour selection period. This method will become a very powerful and influential technique in the field. We contemplate using it to study the potential for A-particle transmission by

fusion of A-bearing cytoplasts with A-negative cells, as well as in transmission of mitochondria and membrane systems from one cell to another in the absence of nuclear transfer. Using these techniques one might inquire whether properties associated with tumor cells such as malignancy and transformation really do require nuclear DNA to transmit their phenotype.

3) In collaboration with Dr. Saverio Ambesi (C:LPHY) we have undertaken to grow rat thyroid cells both from tumors and from the normal thyroid gland. The tumors which have been studied for some years in Dr. Seymour Wollman's (C:LPHY) laboratory have been considered difficult to adapt to cell culture. We have thus far succeeded in adapting one on them (known as 1-5G). Mutants of these cells are being produced in anticipation of using them in hybridizations with normal cells in order to generate cell strains with sufficient genetic diversity to make possible more sophisticated genetic, biochemical, and physiological studies. Part of this problem has involved trying to grow "normal" thyroid cells in culture. Various attempts have been made in the past - some of them apparently successful however, no functional cell strains were produced. Dr. Ambesi has found by using pituitary extract or dibutyrlcyclic AMP (DBcAMP) that normal thyroid cells of the rat may be propagated and subcultured for many cell generations. The persistence of the differentiated state under various experimental conditions will be studied in the coming year. These cell lines and this kind of study are considered to be of great value to the thyroid physiologists. By using thyroidectomized and normal nude mice it should prove possible to examine the status of growth control in these cultured cells relative to hybrid cells, thyroid explants, thyroid tumor cells, and normal thyroid cells grown in culture. This novel approach to growth control experiments is patterned after the recent demonstration by Dr. Gordon Sato's group that the rat liver cell strains, established in our laboratory some eight years ago would grow in nude mice only after partial hepatectomy.

4) Lieber, Sherr, Todaro, Benveniste, Callahan and Coon (PNAS communication) have shown that an entirely new form of C-type virus particle was inducible from a line of heart fibroblasts developed some years ago in our laboratory from an adult male Mus caroli. It is anticipated that this new virus will have far reaching importance in understanding the relationship among these viruses and "normal" and inbred mouse populations.

5) A collaboration has been initiated with Dr. Chris Tan (NIA-GRC (Baltimore)) which will examine the genetics of the suppression of interferon production by reverse and normally segregating human mouse hybrid cells.

It was recently found that a transformed human cell line carried and widely used in our laboratory for hybridizations was a miraculously high (efficient) producer of human interferon. Upon superinduction this cell line produces some 625 times the yield of interferon than had previously been known and furthermore, it is a transformed line and will grow efficiently to high densities - this is the first such line known to produce large amounts of human interferon. A practical source for human interferon for structural, therapeutic, and antitumor studies is now in view. Tan and I have been collaborating on a project of his to map the chromosomes regulating production of human interferon using our reverse segregating human mouse hybrids. This problem may shift its emphasis for a time as we contemplate establishing an interferon production demonstration project.

6) Saving the worst for last: It must be admitted that we have mde no progress whatsoever with the vastly exciting and tantalizing problem raised by our collaboration with Dr. Premkumar Reddy (LCBGY) on the in situ hybridization of immunoglobulin heavy chain message with chromosomes. The original observation stands: the heavy chain message preparations do not hybridize with DNA extracted from purified metaphase chromosomes but do amply hybridize with whole cell DNA. This is an exceedingly powerful observation and will form the basis of the work of our own and other labs for many years to come. The work has progressed little to this point because of the difficulty that Reddy has had in producing the message which is to act as a probe in our studies. Dr. Fred Mushinski (Z01 CBD 03222-07 LCBGY) has joined us in our efforts to get the message preparations to work in Bethesda. We hope within the next few months to have the show on the road again. However a problem of this importance for modern biology must not be halted by this glitch. Other ways exist to study the problem - we know of methods to extract this extra chromosomal DNA and we shall proceed with that and the cRNA made from it as probe in the next year. Furthermore, we have begun to study the ultra-microchromosomes found in many transformed cell lines (particularly mouse lines) for a possible association with the extra chromosomal DNA.

7) A new project has been initiated in collaboration with Dr. Michael Potter (Z01 CBD 05596-07 LCBGY) concerning the reverse translation from known amino acid sequences involved in the (Wu, Kabat) hypervariable regions in human and mouse immunoglobulin. Specifically, I have written a reasonably efficient reverse-translation PL/I program on the IBM 370-165 (168) computers which tests the large numbers of codings possible by the degeneracy of the genetic code. The program was designed to test Potter's theory that potential for secondary structure in the hypervariable regions might be the cause of their propensities for deletion. Potter predicted that the hypervariable

regions would have a large amount of secondary structure pairing - and I thought that it should be the other way around. Remarkably enough it has turned out that these hypervariable regions have very significantly low maximal opportunity for intrastrand basepairing (about 35%). This theory makes predictions about the kinds of amino acids which will be found in the hypervariable regions and if we are lucky could also make unique predictions of the bases in the DNA which code for them. The computer programs will provide fascinating and potentially valuable information about the new discipline of reverse-translation.

Publications:

Dawid, I. B., Horak, I., and Coon, H. G.: The use of hybrid somatic cells as an approach to mitochondrial genetics in animals. Proc. of the XIII Int. Cong. Genetics. Suppl. Genetics 78: 459-471, 1974.

Minna, J. D., Yavalow, J., and Coon, H. G.: Expression of phenotypes in hybrid somatic cells derived from the nervous system. Proc. of the XIII Int. Cong. of Genetics. Suppl. Genetics, October, 1974.

Horak, I., Coon, H. G., and Dawid, I. B.: Interspecific recombination of mitochondrial DNA molecules in hybrid somatic cells. Proc. Natl. Acad. Sci. (Wash.) 71: 1828-1832, 1974.

Dawid, I. B., Horak, I., and Coon, H. G.: Propagation and recombination of parental mtDNAs in hybrid cells. In A. M. Kroon and C. Saccone (Eds.): International Conference on the "Biogenesis of Mitochondria". New York, Academic Press, 1974, pp. 255-262.

Minna, J. D., and Coon, H. G.: Human x mouse hybrid cells segregating mouse chromosomes and isozymes. Nature 252: 401-404, 1974.

Kraemer, K. H., Coon, H. G., Petinga, R. A., Barret, S. F., Rahe, A. E., and Robbins, J. A.: Genetic heterogeneity in xeroderma pigmentosum: Complementation groups and their relationship to DNA repair rates. Proc. Nat. Acad. Sci. (Wash.) 72: 59-63, 1975.

Keyword Descriptors: cell hybridization/ interferon/ immunoglobulins.

Project No. Z01 CBD 05553-07 LCBGY
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structure of mouse myeloma proteins with antibody activity

Previous Serial Number: NCI-5553

Principal Investigator: Dr. Stuart Rudikoff

Other Investigators:
Dr. Michael Potter Z01 CBD 05596-07 LCBGY
Dr. Eduardo Padlan (A:LMB)
Dr. David Davies (A:LMB)
Dr. C.P.J. Glaudemans (A:LC)
Dr. Leroy Hood Cal. Tech.
Dr. Mark Vrana Z01 CBD 05553-07 LCBGY

Man Years:
Total - 2.00
Professional - 1.00
Other - 1.00

Project Description:

Plasmacytomas which produce homogeneous immunoglobulins have been induced in the inbred BALB/c strain of mouse by Dr. Michael Potter. Many of these homogeneous immunoglobulins have been shown to demonstrate antigen binding activity and in fact groups of proteins have been characterized which bind the same antigen. We have been studying three of these groups of proteins in an attempt to 1) determine the structure of the antibody combining site, and the interaction between hapten and antibody at the molecular level and 2) ascertain the type of diversity that may occur among a group of proteins which bind the same well defined hapten.

1. The crystallization of the Fab fragment from the phosphorylcholine binding myeloma protein McPC603 and its general structure at 4.5 Å resolution have been previously described. As a result of the determination of the variable region amino acid sequence and the further refinement of X-ray data, a high resolution model of the antibody combining site has been constructed. We have recently reported a detailed description of the combining site and the detailed interactions between hapten and protein. A cleft has been found at the tip of the molecule which is formed by the three hypervariable regions of the heavy chain and two of the three from the light chain. This surface, composed of

the immunoglobulin hypervariable regions, seems to be universal among antibodies and thus provides the structural basis for antibody function and homology. The hapten, phosphorylcholine has been found to form hydrogen bonds with a tyrosine residue in the first heavy chain hypervariable region and an arginine in the second heavy chain hypervariable region. Other interactions have also been observed with other portions of the hypervariable surface. Data refinement is continuing to further clarify some portions of the structure and to complete analysis of the constant region.

Similar studies are continuing on the crystallized Fab fragments of J539, a galactan binding protein, to permit the detailed comparison of two proteins with different antigen binding specificities.

2. Preliminary comparative sequencing studies of five phosphorylcholine binding myeloma proteins have previously been reported. We have extended these studies and have recently completed the heavy chain variable region determinations on all of the proteins. This comparison has permitted an assessment of the degree of variability among proteins demonstrating the same hapten binding specificity. The differences observed among these proteins have also provided a unique opportunity to assess the possible mechanism involved in the generation of antibody diversity. Studies on the light chains of these proteins are continuing.

A new set of eight proteins with binding specificity for levans has recently been described by Dr. Michael Potter in this laboratory. These proteins have been shown to possess shared serological markers in their variable regions (idiotypes). Sequence analyses of these proteins have been initiated with the objects to 1) ascertain if the variation pattern observed in the phosphorylcholine group is common to other groups with defined specificities and 2) to attempt to define the structural basis of the observed shared serological markers.

As a result of the studies described above work is continuing on the *in vitro* synthesis of antibody molecules with McPC603 as the prototype. Studies of this nature may in the future permit the *in vitro* design, synthesis and/or modification of antibodies with predetermined specificity.

Relationship to National Cancer Plan:

Objective - 6

Approach - 1

Keyword Descriptors:

Immunoglobulins; Three Dimensional Structure; Variable region amino acid sequence; Diversity.

Publications:

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Project No. Z01 CBD 05554-07 LCBGY
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Characterization and measurement of soluble antigens associated with human neoplasms.

B. Study of plasma cell neoplasms in mice and characterization of their immunoglobulin products.

Previous Serial Number: NCI-5554

Principal Investigator: K. Robert McIntire, M.D.

Other Investigators:
Dr. William Adams (Z01 CBD 05554-07 LCBGY)
Dr. Peter Sullivan (Z01 CBD 05554-07 LCBGY)
Dr. Thomas A. Waldmann (C:MET)
Dr. Richard Adamson (C:LCHPH)
Dr. Philippe Sizaret (I.A.R.C. Lyon, France)
Dr. Charles L. Vogel (Emory Univ. Med. Center, Atlanta, Ga.)
Dr. Elliott Perlin (NNMC, Bethesda, Md.)
Dr. Robert Kurman (AFIP, Walter Reed Army Medical Center, Washington, D. C.)

Cooperating Units:
I.A.R.C. Lyon, France
Emory University Medical Center, Atlanta, Ga.
NNMC, Bethesda, Md.
AFIP, Walter Reed Army Medical Center, Wash., D.C.

Man Years:
Total - 4.00
Professional - 3.00
Other - 1.00

Project Description:

A) Characterization and measurement of soluble antigen associated with human neoplasms. Objective - 6, Approach -1

1. Studies of Alpha-fetoprotein (AFP)

A. Quantitation of AFP in human disease: We have continued to use sensitive, quantitative methods to measure the level of serum AFP during the course of therapy for both hepatocellular carcinoma and embryonal cell testicular carcinoma. The apparent usefulness of this marker, together with serum human chorionic gonadotropin (hCG) in testicular neoplasms, has led to tentative trials of using tumor markers in a prospective way to gauge effectiveness of therapy.

In addition to studies of AFP in neoplastic disease we have also been involved in studies of AFP levels in non-neoplastic disease. For these studies the use of a radioimmunoassay is a necessity. AFP has shown to be a useful marker in maternal serum or in amniotic fluid for the early diagnosis of anencephaly and neural tube defects. We are collaborating in such studies and also making our techniques available to other investigators so that the studies may be broadened.

Studies of AFP in a variety of benign liver diseases have demonstrated that serum AFP levels are higher with the more severe forms of tissue damage following infectious hepatitis and that the rise in AFP is temporally related to that of early liver regeneration.

We are also involved in several collaborative studies studying the usefulness of correlating several different markers in the early diagnosis of cancer: AFP, CEA, hCG, polyamines, Regan's isoenzyme, fetal ferritin, etc.

B. Study of AFP in primates: We are continuing to follow a large number of monkeys receiving various carcinogens suspected of hepatoma induction. Following the induction of hepatoma, the marker (AFP) can then be used to predict and follow the effectiveness of therapy. We have also been studying the changes in AFP level following acute liver damage in primates induced by hepatitis virus or by chemical hepatotoxin. These studies seem to confirm our observations in human disease, linking rising AFP levels to the period of liver regeneration.

C. Metabolism of AFP: A new method to purify AFP (antibody-bound affinity chromatography) has provided highly pure AFP which has been labelled with ^{125}I. This purified and labelled AFP shows an identical biological handling and turnover to the native AFP when these are compared. Experiments are now in progress to determine the effect of varying serum levels of AFP on the catabolism of AFP. Monkeys with AFP-synthesizing hepatomas provide the subjects for these experiments. Synthetic rates of AFP production by freshly-obtained biopsy specimens of hepatoma are continuing to be studied. Varying technical problems have been solved, but many remain. At the present it would appear that different hepatomas do synthesize and secrete AFP at different rates; it would further appear that those tumors with the highest synthetic rates result in the animals with the highest serum levels. These relationships will be worked out to determine the relationship between serum AFP and total number of tumor cells.

D. International standard for AFP: During the past year we have participated in two cooperative studies involving up to 13 different laboratories, organized by the W.H.O. under the direction of Dr. Philippe P. Sizaret at the I.A.R.C. The purpose of these studies is to develop a reference standard for any laboratory wishing to quantitate serum AFP. The data are still in the analysis stages but should be completed before the end of 1975 so that the already prepared aliquots may begin serving as an international standard for AFP.

2. Studies of Lung Tumor Associated Antigens: Objective - 5, Approach - 4

A. Efforts to isolate a human lung tumor associated antigen (HLTAA) identified by absorbed rabbit antiserum in immunoprecipitin tests has continued. Antibody bound to Sepharose and used for affinity chromatography was partially successful as was sieving chromatography on Biogel A 1.5. However, both methods left a fairly high concentration of undesirable contamination with other proteins. Acid treatment (pH 1 for 30 min) of saline tumor extracts appeared to remove a large amount of unwanted cellular protein contaminants. DEAE chromatography resolved two antigen peaks with differing electrophoretic mobility, but antigenic identity. The first of these was relatively free of contaminants. Work is proceeding to further isolate the antigen to the point where radio-labelling is possible and a radioimmunoassay can be developed.

B. Attempts have been made to use partially purified HLTAA to immunize rabbits, goats and sheep. So far these have been unsuccessful in producing antiserums with the desired specificity for HLTAA. This work will continue with emphasis on acid extracted antigen and using priming doses of adjuvant alone 10 days to 2 weeks before antigen stimulation.

C. Immunologic detection of cellular antigens can be applied to formalin-fixed tissues by using antibody coupled to horseradish peroxidase and then subsequent development of the peroxidase to fix a benzidine stain. A series of studies is now underway that indicate the presence of HLTAA in all of the 25 primary lung tumors examined. Normal lung tissue does not contain the antigen by this technique, but a few other tumors have been positive. The definition of antigenic specificity by this technique is now underway in collaboration with Dr. Robert Kurman, A.F.I.P.

D. Quantitation of HLTAA: We are working on the technique of inhibition of hemagglutination. HLTAA has been coupled to sheep red blood cells by the method of tannic acid sensitization and has produced hemagglutination with antiserum diluted 1:20,000-1:32,000. The details of specific antigen inhibition of hemagglutination are now being worked on to provide a quantitative assay for following the procedures used in antigen isolation.

B. Mouse Immunoglobulins

1. Immunoglobulin (Ig) markers on the surface of plasma cells: Objective - 3, Approach - 5.

Continued work on aspects of cytophilic host antibody on the surface of tumor cells from BALB/c plasmacytomas, indicate that the presumed host antibody (or antigen-antibody complex) is lost from the cell surface within eight hours following removal from the host into tissue culture. These cells, as well as those from long-term culture, become susceptible to complement lysis following incubation with serum or ascites from tumor-bearing BALB/c mice. Complement-susceptible tumors can also be established by inoculating normal BALB/c mice with non-complement-susceptible cells from long-term culture. We have also found evidence of cytotoxic anti-tumor antibody in the serum of mineral oil-stimulated mice which have developed plasma cell neoplasia. The specificity of the host antibody(s) is as yet unclear.

Keyword Descriptors:

Tumor markers, Tumor associated antigens, Alpha-fetoprotein, Hepatoma, Embryonal cell carcinoma, Chorionic Gonadotropin, Lung Tumor, Surface immunoglobulin, Plasmacytoma.

Publications:

Ravry, M., McIntire, K. R., Moertel, C. G., Waldmann, T. A., Schutt, A. J. and Go, V.L.W.: Carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) in the diagnosis of gastric and colonic cancer. A comparative clinical evaluation. J. Nat. Cancer Inst. 52: 1019-1021, 1974.

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McIntire, K. R. and Sizaret, P.P.: Human lung tumor antigens. Proceedings of the XI International Cancer Congress, Exerpta Medica, Amsterdam, 1975 (in press).

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Project No. Z01 CBD 05596-07 LCBGY
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pathogenesis of plasma cell neoplasia in mice; immunochemical characterization of antigen-binding myeloma proteins; genetic basis of immunoglobulin diversity

Previous Serial Number: NCI-5596

Principal Investigator: Michael Potter, M. D.

Other Investigators:
Drs. R. Asofsky and H. Morse (I:LGAR)
Dr. R. Lieberman (I:LCI)
Dr. S. Rudikoff (Z01 CBD 05553-07 LCBGY)
Dr. Mark Vrana (Z01 CBD 05596-07 LCBGY)
Ms. E. B. Mushinski (Z01 CBD 05596-07 LCBGY)
Dr. David R. Davies (A:LMB)
Dr. C. P. J. Glaudemans (A:LC)
Mr. Michael Cancro (GW) Univ. of Maryland

Man Years:
Total - 4.0
Professional - 1.0
Other - 3.0

Project Description:

1. Pathogenesis of plasma cell tumor development.

Plasma cell tumors can be induced in 60 to 70% of BALB/c mice by the intraperitoneal injection of various mineral oils or pure hydrocarbons (e.g. pristane). With this model system several factors appear to participate in plasmacytoma development: 1) Genetic susceptibility, revealed by the strain susceptibility of inbred strains BALB/c and NZB and the resistance in most other strains (C57BL, DBA/2, C3H, ALN); 2) An abnormal tissue microenvironment - the mineral oil granuloma which promotes plasmacytoma development; 3) An initiating factor which could be due to replication of an intrinsic latent virus or a spreading somatic infection by an endogenous virus; 4) Antigenic stimulation.

A. Genetic factors in mineral oil induced plasmacytoma formation.

We have been trying to find a rapid method for inducing plasmacytomas to facilitate identifying genes in BALB/c mice associated with susceptibility.

A rapid, but somewhat inefficient means of plasmacytoma induction has been developed using a single injection of pristane (conditioning) followed by infection with Abelson's murine C-type leukemia virus (MLV-A). When mice are conditioned for 40 days or so the incidence of plasmacytomas ranges from 15-20% in the first 100 to 150 days post-virus infection. We have spent considerable time on determining the interval of time between conditioning and infection. Short periods of 10 days or less do not appear to be as effective as 30- to 50-day periods suggesting conditioning stimulates target cell production. Experiments are in progress to determine the interval. These studies require production of large quantities of potent virus. We have encountered some difficulties, but now have several pools. The infecting dose of virus also appears to be a factor in plasmacytoma induction - too potent virus preparation over-produces lymphosarcomas; a weak preparation produces too few, thus virus preparations need to be titered. These complications have slowed progress during the year.

Only a few inbred strains are susceptible as adults to MLV-A infection; these include the plasmacytoma-susceptible BALB/c(C) and the plasmacytoma-resistant DBA/2(D) and CDF_1 hybrids. Preliminary data indicate DBA/2 and CDF_1 are resistant to plasmacytoma induction, thus we have a potential system for determining the genes associated with sensitivity. This however, is very inefficient in its present state, and will require 1) development of methods for increasing plasmacytoma yield, and 2) recombinant-inbred (RI) strains of mice derived from crosses of BALB/c and DBA/2. We are currently producing such RI strains, emphasizing lines, with minimal numbers of DBA/2 genes.

C57BL and (BALB/c x C57BL)F_1 are resistant to MLV-A infection as adults. Treatment of mice with antilymphocyte serum during infection with MLV-A (exps. done with L. W. Law) produced a high incidence of lymphosarcomas (with a longer latent period) was obtained in ALA-treated, MLV-A infected pristane conditioned C57BL. No plasmacytomas were obtained in the latter, however, again indicating genetic resistance to plasmacytoma induction.

B. Abnormal tissue microenvironment

Continuing studies on the role of the oil granuloma microenvironment are being carried out with Michael Cancro, a graduate student at the Univ. of Maryland. Essentially it has been found that the conditioning effect of i.p. pristane can be abrogated by treatment of the mice with hydroxycortisone. Hydrocortisone is known to block the formation and ingress of adherent peritoneal exudate cells (APEC). In general we hypothesize APEC produce growth factors for plasma cells and that early plasmacytomas require this factor for growth.

Mr. Cancro is also developing an assay method for plasmacytoma growth and viability enhancement *in vitro*, using sustained immunoglobulin production by an unadapted primary plasmacytoma as an index. Initial studies have indicated the feasibility of the assay. Conditioned medium from cultured adherent peritoneal exudate cells have stimulated viability enhancement. We are intensively investigating this phenomenon.

II. Immunochemistry of myeloma proteins. Genetic basis of diversity. Structure of combining site of antigen binding myeloma proteins.

A. Structure

Groups of BALB/c myeloma proteins that bind phosphorylcholine. β1→6D galactan, α 1→3, α 1→6 dextran, β 2→6 fructosan, DNP have been collected and studied both structurally and antigenically.

Structural studies in collaboration with Dr. Stuart Rudikoff and Dr. David Davies' group involve determination of primary structures, and 3-dimensional structures by X-ray crystallography. A 3.1 Å electron density map of the phosphorylcholine binding myeloma protein McPC603, based on complete sequence of the V_H and 80% of the V_L has been completed. The crystal was made with the ligand in site and this permits the definition of the binding site. A second crystal is being prepared with J539. This information provides the means for a new approach to immunochemistry.

Essentially, the Ig combining site is localized at one pole of the Fv, in a continuous surface bounded by the hypervariable segments from the V_L and V_H. Since the internal architecture of V_L and V_H domains in both mouse and man is extremely similar, by knowing the 3-dimensional structure of the binding site it will become possible to modify the structure of antibodies. We are continuing to screen for new antigen binding myeloma proteins and test each for their ability to crystallize.

In the last year the levan binding myeloma protein system has been intensively investigated; fourteen proteins have been collected and characterized. These have been divided into 2 groups, a large group of proteins that bind β2,1 linked fructosans and a smaller group with specificity for β2,6 linked fructosans. Dr. Mark Vrana is isolating these proteins and determining their amino acid sequence.

In collaboration with Dr. Rose Lieberman we have studied the individual and cross-specific idiotypes of these proteins. Each protein has an individual idiotype and one or more cross specific determinants.

The large number of these determinants suggest and the preliminary sequence data indicate a degree of variability of closely related proteins that may be the result of a form of somatic variation. We are actively pursuing these studies.

With Ms. E. B. Mushinski we are continuing our work with the galactan proteins looking specifically for cross-specific idiotypic determinants which have been found using both homologous and heterologous systems. In addition we are testing each of these antigenic systems for possible use as a genetic marker.

We continue to develop, characterize and produce Ig-congenic strains of mice. We are inducing plasmacytomas in these mice and screening for antigen binding activities.

III. Immunoglobulin synthesis

In collaboration with Ms. Janet Pumphrey, Drs. H. Morse, and R. Asofsky we are screening plasmacytomas for double-secretors. Two transplantable double secreting tumors have been identified.

In collaboration with Dr. E. Premkumar Reddy we have shown that Ableson lymphosarcomas produce membrane bound immunoglobulin. These tumors are being screened for their ability to make rosettes with SRBC coupled with various antigens.

Keyword Descriptors:

Plasmacytoma induction, Abelson murine leukemia virus, antigen binding site structure, mineral oil, pristane, immunoglobulin genetics, galactan-, levan-, phosphorylcholine-binding proteins.

Publications:

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Finlayson, J. S., Potter, M., Shinnick, C. S., and Smithies, O.: Components of the major urinary protein complex of inbred mice: Determination of NH_2-Terminal sequence and comparison with homologous components from wild mice. *Biochemical Gen.* 11: 325-335, 1974.

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Editor: Warner, N. L., Potter, M. and Metcalf, D. (Eds.): Multiple Myeloma and Related Immunoglobulin-Producing Neoplasms. Geneva, Workshop on Multiple Myeloma, UICC Technical Report Series, 1974, Vol. 13, 88 pp.

SUMMARY STATEMENT
ANNUAL REPORT
DERMATOLOGY BRANCH
DCBD, NCI
July 1, 1974 through June 30, 1975

The Dermatology Branch conducts both clinical and basic research studying the etiology, diagnosis and treatment of malignant diseases involving the skin and the host's response to these diseases. The Branch also serves as Dermatologic Consultant to all other services of the Clinical Center (approximately 800 patients are seen in consultation each year) and to NIH employees through the Employee Health Clinic. The senior staff of the Branch currently includes the Branch Chief, 5 Senior Investigators, 1 Staff Fellow, 1 Visiting Associate, 2 Visiting Fellows, 7 Clinical Associates, and 2 Guest Workers. The Dermatology Branch has made significant contributions to the objectives of the National Cancer Plan. The main research achievements of the Dermatology Branch for the past year are as follows:

T-Cell Leukemia (Sezary Syndrome) Another major area of research in the Dermatology Branch is the study of the Sezary syndrome, a lymphocytic leukemia of the T-cell type. The Sezary syndrome is a lymphomatous disease characterized by intense generalized reddening and exfoliation of the skin, along with leukemic cells circulating in the blood. Findings of the past year include:
(1) Three patients with cutaneous T-cell leukemia have now been treated by leukapheresis. One patient, who presented at the time of initial therapy, with tumor stage mycosis fungoides superimposed on a generalized erythroderma and with a lymphocytosis of more than 200,000 per cu mm, has been adequately managed for the last 18 months by leukapheresis alone. Two other patients have also significantly benefited from leukapheresis. One patient is presently undergoing extracorporeal irraditation to determine whether this is a more efficient means of managing patients with lower peripheral blood lymphocyte counts;
(2) Cells from one patient were hypotonically lysed following leukapheresis and membrane components were conjugated to a cyanogen bromide activated sephadex column. This column was subsequently used as an immuno-absorbent column to remove and then concentrate the anti-T-cell activity in plasma from patients with lupus erythematosus. Such a column is of major value in the study of all diseases with anti-T-cell activity and the active factors are themselves valuable reagents;
(3) Serum from each of 6 tested patients with cutaneous T-cell leukemia contained large amounts of macrophage inhibitory factor, which could be traced back to the leukemia cells. The serum of these patients therefore represent reservoirs from which this material can be isolated and characterized. Of clinical significance, the presence in the serum of a factor which has a profound effect on circulating monocytes may be at least in part responsible for observed anergy in the patients and their susceptibility to infection;
Lymphomatoid papulosis is an unusual disease in which although malignant looking cells are found in skin tumors, these tumors ordinarily disappear

spontaneously. We have studied patients with this disease, clinically, microscopically and immunologically. Findings have shown that the infiltrating cells in this disease are T-lymphocytes and that nuclear irregularity of the cells of the dermal infiltrates is significantly greater than infiltrating lymphocytes in delayed hypersensitivity reactions. Also it has been found that patients with the syndrome demonstrate normal reactivity to intradermally applied antigens and normal in vitro stimulation of their lymphocytes by mitogens.

Xeroderma Pigmentosum: This rare autosomal recessive dermatologic disease is characterized by pigmentary changes, telangiectasia, atrophy and tumor formation in response to even minimal sun exposure. Tumors which commonly occur in XP patients (basal and squamous cell carcinomas, malignant melanomas etc.) are histologically identical to those found in otherwise normal persons. A defect (presumably in an endonuclease) in repair of UV damage to DNA has been found in XP cells. The NCI, Dermatology Branch, has the largest series of XP patients on whom complete photobiological, clinical and laboratory evaluation have been performed. By complementation analysis of various cell lines in XP in both our collection and those in the laboratories of Prof. Bootsma, of Rotterdam, it has been found there are at least 5 XP complementation groups suggesting that defects arising in any one of at least 5 genetic loci can give rise to XP. In addition it has been found that the neurological abnormalities attributable to XP occur only in patients in complementation groups A and D: the neurological abnormalities may therefore be caused only by the specific mutations that are associated with these complementation groups. Also, the complementation groups are found to correlate with the magnitude of the repair defect as reflected in the rate of UV-induced thymidine incorporation. In cooperation with Dr. Rufus Day it has been determined that complementating fibroblasts can restore biologic function to UV-damaged DNA. In addition we have become interested in the possible relationship of ageing and the ability of cells to repair damaged DNA. In order to determine the effect of the number of cell divisions which occurs in culture on the ability of cells to repair UV-induced damage to DNA, we are currently comparing rates of UV-induced DNA repair in the same cell lines at different passage numbers using cells from our XP patients. Also, long-term lymphoblastoid lines from XP patients peripheral blood have been established, making it possible to obtain large quantities of XP cells for biochemical analysis and hopefully enabling the characterization and purification of defective DNA repair enzyme or enzymes in XP cells. Along with Dr. Zimmerman we have determined that the levels of DNA ligase activity in the XP variants are normal. Studies are also being conducted to determine the effect of different culture conditions on the rate of UV-induced unscheduled thymidine incorporation in fibroblasts. Finally, studies are being performed to determine the sensitivity of fibroblast strains to UV radiation. Different sensitivities in different complementation groups have been found. Understanding the relationship between DNA repair deficiency and tumor development in XP patients should elucidate the role of DNA repair, hopefully leading to prevention in other humans those cancers which may be due to certain DNA-repair related chemical and physical carcinogens as well as ultraviolet radiation.

Malignant Melanoma. Malignant melanoma is a highly malignant tumor in which production of a specific differentiated cell product, melanin, not only continues but is often increased. Melanin granules in tumors are morphologically distinguishable from those found in normal cells. The Dermatology Branch is investigating melanogenesis and its regulation in normal and malignant cells to determine the relationship of this process to melanoma formation. It has been found that the enzyme peroxidase is not responsible for melanin biosynthesis, although this had been suggested by some workers. Also, it has been found that tyrosinase activity which is the single enzyme responsible for melanin synthesis is under allosteric control and that phospholipids may play a part in the expression of the enzyme activity. It has also been found that this allosteric regulation is altered in murine melanoma; this perhaps explains the atypical menanosome formation in melanoma tissues. In view of the differences noted between the regulation of tyrosinase in normal and melanoma tissues it is felt that at least one of the primary differences between the melanogenic capabilities of these two tissues has been revealed. That these controls are operative at the post-translational level is informative. It is hoped that the cause of enzymatic differences in malignancies will provide insights into the nature of neoplastic transformation. Further it has also been found that the melanosome in normal mouse tissues are composed of multiple proteins, many which are loosely bound even when extracted; probably they constitute proteins of the limiting membranes of the organelle. Still another protein is of lower molecular weight and is tightly bound within the granule, probably constituting the structural protein; it appears to be the protein which complexes with the melanin polymer. Melanosomal proteins from melanoma tissues vary in structure from those of normal tissues. Several of the proteins seem to be missing completely from these granules which are also structurally distinguishable from normal granules by electron microscopy. Perhaps, more importantly, many of the proteins in melanoma melanosomes are unique and are not found in normal melanin granules. This has been found to be the case in human melanoma as well. These observations concerning the aberrant biochemical characteristics of tyrosinase and structural proteins in the melanoma melanosome indicate the control of melanogenesis is in some manner affected by cancer formation. These studies are being extended to human melanomas in an attempt to characterize the level in which these disoriented control mechanisms operate, hoping that further insight into such controls, be it at the level of replication transcription, translation or post translation, will provide clues as to the level in which carcinogenic information is expressed.

Isolation and Chemical Characterization of Human Epidermal Tonofilaments. Tonofilaments are a major component of the epidermal cell although little or nothing is known about their chemistry of function. Therefore it seems important to know more about these structures in order to understand normal epidermal cells as a base line for studying malignant epidermal diseases, such as basal cell and squamous cell carcinoma, probably the two most common cancers occurring in man. It has been found that intact tonofilaments of bovine epidermis are extractable by a simple buffer system and can be purified by differential centrifugation and gel electrophoresis. The tonofilaments appear to be composed of 7 different peptide chains. These chains have been further classified into 3 groups. These isolated peptides have a propensity for aggregating into filamentous structures which make them accessible to study by electron microscopy and X-ray diffraction which is being done. In addition skin from patients with benign and malignant lesions have been studied using the same system in an attempt to detect whether there are any differences in these tonofilament polypeptide chains. One of the major problems in studying malignancies of epidermis in the past has been the lack of a suitable biochemical marker. Tonofilaments are a prominent component of all epidermal cells. Elucidation of their chemistry, structure, and synthesis might aid in determining the nature of human epidermal cancer.

Scanning and Transmission Electronmicroscopy. Structural parameters of diseases are being studied with a wide range of optical methods comprising light microscopy, transmission electron microscopy, and scanning electron microscopy. These observations provide the most detailed descriptive morphologic data available with the current technology and gives direct experimental evidence for the physiologic roles of certain cells and organelles, and helps in evaluating the purity and sampling of cells or cell components being studied by other methods. The development, behavior, fate and pathology of leukocytes and epithelial cells are a primary concern of our laboratory. These cells play an essential role in normal and diseased skin and are uniquely suited for experimental studies. It has been found that normal human peripheral lymphocytes almost invariably appear villus when fixed in suspension by our standard procedures. Also by a variety of methods it has been found that there is little difference between T-lymphocytes and B-lymphocytes according to their surface structure, in opposition to what has been reported by others. In an effort to discover and develop new methods of interpretive criteria for the application of the scanning electron microscope to a wide variety of soft biologic tissues, attempts have been made to improve survey and sampling methods. A human tumor cell line (HBT-3) has been studied, and it has been found that during the first 24 hours after seeding cell structure is profoundly influenced in distinctive ways by kind of culture medium, the underlying substrate, and the concentration of supplemental serum in the medium. Further, Cinephotomicrographic studies in conjunction with scanning electron microscopy of the same cultured mouse cells provide the best evidence to date that surface morphology is independent of the stages of the cell cycle. It has been determined that one cannot predict the mitotic behavior of cells by their topography; also it has been determined that one cannot as yet determine whether or not cells are neoplastic or benign by their cell topography.

Immunopathologic Mechanisms Involved in Blistering Skin Diseases. This is a new area which has just been developed with the arrival of a new senior investigator in the Dermatology Branch. Studies of blistering diseases will employ the methods of direct and indirect immunofluorescence, cell mediated cytotoxicity, and immunochemistry for identifying immunoglobulins and immune complexes. Other techniques to be used include radioimmunoassays, and immunoperoxidase at the electron microscope level. It is hoped that a basic understanding of immunologic injury in various autoimmune disease states affecting the skin and other organs will prove important in interpreting and furthering current concepts in self-recognition.

Animal Models for Genetic Human Disease. A rat has been found, which on the basis of an autosomal recessive hereditary defect develops spontaneous and traumatic blisters. Also, the rat loses its hair at an early age. It has been found that there is a suprabasilar cleft which explains the blister formation. At the moment, although there is resemblence to several human genetic diseases, this animal model does not seem exactly matched with any known human genetic blistering disease. However, it is the first animal model that has been described for blistering disease and further studies might provide information as to how normal epidermal cells are held together.

Epidermal Differentiation and Miscellaneous Therapies for Skin Disease. It is known that Vitamin A acid alters epidermal cell differentiation resulting in inhibition of keratinization. Biochemical and scanning electron microscopic studies have been performed to view these changes in chick embryos. Vitamin A is known to inhibit carcinogenesis and to be of value in the treatment of some malignancies. The mechanism of action of Vitamin A in inhibiting keratinization may be related to its antineoplastic activity. It has been found that Vitamin A inhibits formation of several polypeptides, while promoting the formation of a specific mucopolysaccharide.

There are now underway studies to evaluate the safety and effectiveness of topical α-hydroxy acids in the treatment of ichthyosis, psoriasis, Darier's disease and other disorders of keratinization. There have been varying reports on the effectiveness of these agents in skin disease. This study is being carried on further to identify which diseases respond most readily to these new agents.

Topical BCNU and CCNU are effective in treatment of some skin cancers. However because of the absorption into and through the skin as evidenced by bone marrow toxicity, the use of these drugs at the moment are precluded and an attempt is being made to discover other topical agents which would be effective in treatment of skin cancer without concomitant toxic side effects.

Study of Cell Mediated Immunity and Contact Sensitization in Human Skin. The sensitization of human skin with the chemical Dinitrochlorobenzene (DNCB) has become a very frequently used means of testing the ability of humans to generate a cell mediated immunity response. Using a method developed in our laboratory for *in vitro* testing of cell mediated immunity, there has been a quantitative correlation found between *in vivo* skin test reactivity to DNCB and in vitro specific blast transformation which represents in humans at present the best *in vitro-in vivo* correlation for cell mediated immunity. Further studies have indicated that a factor is released by sensitized lymphocytes in culture which is capable of recruiting and inducing blastogenesis in lymphocytic cells from non-sensitized subjects. This particulate lymphokine is completely removed by filtration through .45 micromillipore filters. Further biochemical studies of this factor are being attempted. It has also been demonstrated that in a series of DNP-containing amino acids all are capable of inducing specific blast transformations. These findings should be of importance in eventually understanding the mechanisms of classical contact sensitivity which represents a form of cell mediated immunity seemingly related to the mechanisms involved in graft rejection and tumor specific immunity. Finally these methods will be used to pinpoint defects in a wide variety of diseases in which there are abnormalities in cellular immunity.

Project No. Z01 CBD 3613-02 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Extraction, Isolation and Characterization of Keratohyalin Granules

Previous Serial Number: Same

Principal Investigator: Walter G. Klingler, M.D.

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total	1.4
Professional:	0.7
Other:	0.7

Project Description:

Objectives:

1) To isolate and characterize human and rat keratohyalin.

2) To demonstrate the histochemical, ultrastructural and biochemical properties of human and rat keratohyalin.

3) To elucidate similarities and differences of keratohyalin from various animal species by isolation and subsequent biochemical and immunological characterization.

4) To develop a short-term organ culture system for bovine epidermis.

5) To isolate bovine keratohyalin synthesized in organ culture utilizing tritiated amino acid precursors as radiochemical markers.

6) To monitor the effect of various pharmacologic agents on the synthesis of bovine keratohyalin, a specific epidermal marker protein in short-term organ culture.

7) To study the physiology of bovine keratohyalin synthesis in organ culture by employing metabolic blocking agents (e.g. puromycin) and agents which may stimulate protein production (e.g. dibuitryl cyclic AMP).

8) To apply the short-term organ culture of bovine epidermis to address basic questions regarding function of keratohyalin.

The Objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

1) Human and rat tissue are fixed routinely and studied histochemically and ultrastructurally. Extraction patterns are determined as previously described using serial dilutions of various salt solutions.

2) Human and rat epidermis are prepared and extracted in 1.0 M potassium phosphate buffer. Aggregates are formed by dialysis against distilled water; these aggregates may be fixed routinely and studied histochemically and ultrastructurally or solubilized in sodium decyl sulfate and subjected to disc gel electrophoresis.

3) Bovine epidermis is prepared as previously described in the presence of media 199 containing penicillin and streptomycin. Epidermis is minced into 1 mm cubes and then placed into a metabolic shaker. Tritiated precursors and pharmacologic agents (when desired) are then added to the media. Incorporation is terminated at various time periods. Bovine keratohyalin is isolated by high-salt in vitro aggregation methods and specific activates are determined.

Major Findings:

1) The extractability of rat keratohyalin vs strength of phosphate buffer extractant has been clearly established and is comparable to bovine keratohyalin.

2) A protein reaggregate obtained following dialysis of a phosphate buffer extract of rat skin has been studied histochemically, ultrastructurally, and biochemically.

3) This reaggregate from rat skin is similar in many respects to bovine keratohyalin, but interesting ultrastructural and physical-chemical differences between bovine and rat keratohyalin have also been established.

4) Short-term organ culture of bovine epidermis with subsequent chemical isolation of keratohyalin is feasible. Significant parameters - culture time, tissue/media rates, tritiated precursor amino acid/tissue ratio - have been studied. Parameters were then idealized to make the organ culture system reproducible and statistically significant.

5) Bovine epidermis in organ culture will vary the synthesis (as monitored by specific activity) of bovine keratohyalin depending on the pharmacologic agent added to the media, but further work is needed to establish statistical significance.

Significance to Cancer Research:

The epidermis is an invaluable asset in studying carcinogenesis because of the epidermis' availability and its rapid turnover of cells even under normal conditions. As an easily identifiable extractable protein keratohyalin can be used as a biochemical marker, and indeed, is absent in basal cell carcinoma, squamous cell carcinoma and psoriasis. Organ culture of bovine epidermis has the potential of being an effective screen to study both carcinogens and drugs.

Proposed Course of Project:

The project has been terminated.

Keyword Descriptors:

Isolation, characterization of keratohyalin granules, human and rat keratohyalin, histochemical, ultrastructural and biochemical properties of human and rat keratohyalin, short-term organ culture, carcinogenesis

Honors and Awards: None

Publications:

1) Klingler, W.G., Montague, P.M.: Extraction and Partial Characterization of Rat Keratohyalin Granules. Publication pending.

2) Elias, P.M., Klingler, W.G., Montague, P.M.: Kinetics of In Vitro Bovine Keratohyalin Synthesis. Publication pending.

Project No. Z01 CBD 3625-04 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epidermal Macromolecular Synthesis in Cultured Embryonic Chick Skin and in Normal and Diseased (Benign and Malignant) Human Skin: A Biochemical, Autoradiographical Study With Particular Emphasis on the Effects of Vitamin A

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: F. Ulrike Lichti, Ph.D., and Peter M. Steinert, Ph.D.

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total:	1.28
Professional:	0.38
Others:	0.90

Project Description:

Objectives:

1) To investigate cutaneous macromolecular biosynthesis, and to define the effect of vitamin A in inhibiting keratinization, in embryonic skin, normal adult skin, and in benign and malignant lesions.

2) To define certain dermatologic disorders at the biochemical level.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

1) 0.2-0.4 mm thick, Castroviejo keratotome slices of normal and diseased human skin. Skin from patients with Darier's Disease (NCI-3643) has been used to date.

2) 3-4 mm punch biopsies of normal and diseased human skin (NCI-3643).

3) Tarso-Metatarsal skin from 13-21 day chick embryos.

Methods Employed:

Chick embryo skin or skin from patients with Darier's Disease was incubated both with and without Vitamin A in a water bath shaker at 37°C for 1/2 to 6 hours in the presence of labelled precursors. Prior to exposure to radioactivity, chick skin was maintained in organ culture with and without vitamin A for 0-3 and occasionally up to 10 days.

After incubation, specimens were prepared for biochemical extraction and analysis. Various macromolecular fractions (DNA, RNA, protein and glycoprotein) were isolated using standard methodology. The specific activities of these fractions were determined, and separation and characterization of individual molecular species was defined using polyacrylamide gel electrophoresis. Quantitative and qualitative changes in response to experimental design could be readily followed by banding patterns assayed both spectrophotometrically and by liquid scintillation counting.

Major Findings:

We have studied the effects of vitamin A on embryonic chick skin at the biochemical level. DNA synthesis as measured by ^{3}H-Thymidine incorporation declines progressively and becomes negligible by the 6th day after explanation. In contrast to other systems, vitamin A does not stimulate DNA synthesis. On the other hand, overall rate of protein and RNA synthesis is not affected by placing skin in culture. At the microscopic level, treatment with vitamin A causes mucous metaplasia, involving the inhibition of normal keratinization and the simultaneous stimulation of mucin production. Electrophoretic analysis indicates that synthesis of several major polypeptides is turned off by vitamin A administration (this would account for the inhibition of keratinization very nicely), but to date, we have been unable to find the stimulation of any protein which might represent the prosthetic group of mucin (a glycoprotein). On the other hand, when H^{3}-fucose (one of the sugars in epithelial mucins) is used as precursor, it can be seen, again with the aid of gel electrophoresis, that several glycoproteins are decreased in synthesis with vitamin A administration, while one low molecular weight glycoprotein (which is absent in normal tissue) is synthesized very actively. ^{35}S-sulfate incorporation into macromolecules of the epidermis, presumably sulfated micropolysaccharides, is also stimulated by vitamins. While further work is needed, these results would seem to indicate that vitamin A causes mucus metaplasia primarily by inhibiting keratinization, and at the same time allowing mucin formation to occur, presumably by the glycosylation of preexisting protein in the chick epidermal cell.

Preliminary electrophoretic analyses have indicated that several major protein components found in normal skin are lacking in involved skin of Darier's. This data, when considered with the overall hypometabolism found in Darier's patients, might indicate that certain metabolic processes are inhibited in these patients. In response to vitamin A treatment, although the overall rate of protein synthesis was only slightly increased in Darier's skin, whatever metabolic block was functioning in the suppression of the several missing proteins, was effectively removed, and substantial synthesis of these proteins began in these tissues. It has been known for some time that occasional patients with Darier's Disease respond to topical and systemic therapy with vitamin A. This study may provide the first clue as to the mechanism of action of vitamin A in the treatment of Darier's Disease. The identity of these critical proteins remains to be established. We are currently studying the synthesis of the various RNA's in these tissues, and the response of their synthesis to vitamin A treatment, in an effort to determine at what level these inhibitory factors and the depression mechanisms are located. These studies, it is hoped, will indicate the metabolic abnormality in Darier's Disease, and hopefully, an effective way to correct it.

Our results indicate that vitamin A acts at the level of cellular control mechanisms, and, at least in the case of embryonic chick skin, normal and Darier's human skin, causes basic redifferentation of the cell at the molecular level.

Significance to Cancer Research:

Since carcinogenesis is an instance of altered differentiation, studies of vitamin A-induced redifferentiation, both in human and chick skin, may serve as an excellent model for investigations of cellular control mechanisms which relate to carcinogenesis. The fact that carcinogen metabolism and carcinogenesis are influenced very markedly by vitamin A deficiency directly links research on vitamin A epithelial effects to cancer research.

Proposed Course of Project:

In chick skin, continued biochemical analysis will be done in order to more accurately characterize the epidermal mucin formed, and the mechanism by which vitamin A inhibits normal epidermal protein synthesis and stimulates mucin biosynthesis.

In Darier's Disease, more patients will be studied to confirm our preliminary observations, and to characterize the major epidermal proteins that are lacking in diseased skin. Skin from normal volunteers may be needed for comparative analysis.

Keyword Descriptors:

Cultured Embryonic Chick Skin, Vitamin A, Inhibition of Keratinization, Darier's Disease, DNA synthesis, Epithelial Mucins, Gel Electrophoresis,

Altered Differentiation.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 3630-05 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Vitamin A Acid Induced Mucous Metaplasia of Embryonic Chick Metatarsal Skin in Organ Culture: A Histochemical and Scanning and Transmission Electron Microscopic Study

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Bruce K. Wetzel, Ph.D., Peter M. Elias, M.D., F. Ulrike Lichti, Ph.D., Stuart Yuspa, M.D.

Cooperating Units: Experimental Pathology Branch, DCCP

Man Years (July 1, 1974 through June 30, 1975)

Total:	0.60
Professional:	0.50
Others:	0.10

Project Description:

Objectives:

1) To investigate the mechanisms governing epidermal cell differentiation.

2) To morphologically investigate the mechanism by which vitamin A alters epidermal cell differentiation with resultant inhibition of keratinization.

3) To determine if epidermal cells are capable of both mucin and keratin production, or whether the two processes are mutually exclusive.

4) To study the early changes of vitamin A induced mucous metaplasia at the ultrastructural level.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

Metatarsal shank skin (devoid of feathers) is obtained from 13-14 day chick embryo.

Methods Employed:

Chick metatarsal skin is cultured in vitro and stimulated to undergo mucous metaplasia by vitamin A acid at varying doses. Specimens are taken at varying time intervals and evaluated for mucin production by the use of special strains, such as PAS (with and without diastase), Alcian Blue, Toluidine Blue, and Colloidal iron. Specimens are also fixed in glutaraldehyde for subsequent examinations under the transmission and scanning electron microscopes. In addition, the scanning electron microscope is being used to study in ovo skin scale and feather development, and their inhibition by vitamin A acid in vitro. The effect of retinyl acetate on the morphology of mouse epidermal cells in culture is also being studied.

Major Findings:

1) Using 30 international units of vitamin A acid per milliliter of culture media, it was found that mucous metaplasia occurred with 2 days in in vitro incubation. Maximum metaplasia took place for 6 days.

As little as 1 I.U. produced metaplasia in 6 days. Metaplasia was inhibited or reduced greatly at room temperature, or in the absence of serum. We have studied specimens of chick skin harvested prior to 2 days of incubation and treated with low dosage vitamin A in order to determine the earliest mechanism involved in mucus metaplasia. Ultrastructurally, we found no specific mechanism operative, other than increased amounts of rough endoplasmic reticulum and polyribosomes indicating increased protein synthetic activity.

2) Microscopically, the control specimen keratinized normally with the formation of thick bundles of tonofilaments, keratohyalin granules and a multi-layered stratum corneum. Vitamin A acid treated specimens did not undergo keratinization. There was no tendency of the columnar basal cells to flatten as they approached the surface. Neither stratum corneum nor keratohyalin granules were formed. Tonofilaments were present in small, fine bundles. In addition, there was increased folding of the nuclear membrane, and increased amounts of rough endoplasmic reticulum and polyribosomes. Mucin was present in the cytoplasm as membrane-bound electron dense material. Other transmission E.M. findings included desmosomal cleavage, formation of tight junctions, and basement membrane gaps with elongated processes of epidermal cells extending into the dermis between the gaps. The germinative cells of the epidermis are small, darkly staining, and increased in number. The more superficial spinous cells are large and lightly staining suggesting a distinct and direct effect on germinative cells.

3) In addition to mucin formation there seems to be a glandular

metaplasia in the epidermis with formation of acini. This morphologic change has not been demonstrated previously. Our standard system now utilizes 13-14 day old chick embryos treated with 20 i.u. vitamin A acid in Eagle's Minimal Essential Media with 5% fetal calf serum. Cultures are harvested at 2-3 days. Sixteen-day and older chick embryo skin manifested a selective basal cell layer response to vitamin A.

4) Vitamin A induced mucus glandular metaplasia was inhibited by the addition of puromycin or cycloheximide to the culture medium; actinomycin D, hydroxyurea, and colchicine failed to inhibit this process. Thus suggesting that protein synthesis may be necessary for metaplasia, while mitosis, and DNA and RNA synthesis may not.

5) Scanning electron microscopy revealed that metaplastic changes were visible as early as one day after vitamin A exposure. Individual epidermal cells lost attachments to their neighbors, became spherical, and developed surface villi. Selective basal cell layer effect was observed in 17-day chick embryos treated with vitamin A. Although the superficial stratified squamous layer of the epidermis was unresponsive to vitamin A, it was thrown into large folds by the hyperplastic masses of underlying metaplastic cells.

6) Chick skin scale formation is inhibited by vitamin A acid.

Significance to Cancer Research:

1) Vitamin A is known to inhibit carcinogenesis and to be of value in the treatment of malignancy. The mechanisms of action of vitamin A in inhibiting keratinization may be related to its antineoplastic activity.

Proposed Course of Project:

Continued ultrastructural examination of the above material.

Keyword Descriptors:

Vitamin A Acid, Mucous Metaplasia, Embryonic Chick Metatarsal Skin, Organ Culture, Scanning and Transmission Electron Microscopy, Epidermal Cell Differentiation, Inhibition of Keratinization.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 3631-05 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Treatment of Skin Cancer with Topical BCNU, and CCNU

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1974 through June 30, 1975)

Total:	.01
Professional:	.01
Other:	0

Project Description:

Objectives:

To evaluate the therapeutic effectiveness and toxicity of BCNU and CCNU administered topically to the skin surface of the above patients.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (along and in combinations) in the cure and palliation of cancer.

Agents:

0.1%, 0.2% and 0.4% Bischloroethyl-nitrosourea; and 0.1%, 0.4% and 1.0% chloroethyl-nitrosourea, in 30% liquid petrolatum, 70% white petrolatum.

Material:

1) Basal Cell Carcinoma
2) Nevoid Basal Cell Carcinoma Syndrome
3) Xeroderma pigmentosum with cutaneous carcinoma

Methods Employed:

1) Patients must have pathologic confirmation of the clinical diagnosis.
2) They must have normal hematologic, hepatic, and renal function.

3) Lesions on eyelids and canthi are not included.
4) Patients are cautioned to avoid sun.
5) Ointments are applied twice.
6) Laboratory tests are done before, during, and 6 weeks after therapy.
7) Serial photographs are taken.

Major Findings:

1) A total of 12 patients were included in this study. Nine received only BCNU ointment. One received only CCNU ointment; the remaining two received CCNU ointment initially and were given BCNU later as a second course of therapy. Five of the 12 patients were treatment failures by other modalities, including surgery and topical 5-FU in propylene glycol. Four of the patients had had X-ray therapy of their acne earlier in life. Four other patients had moderate to severe actinic skin. Two patients had the basal cell nevus syndrome; one had xeroderma pigmentosum. Most patients had lesions of the exposed areas of face and neck. Many patients also had lesions on the trunk and extremities. None of the 12 had the standard variety of BCE histologically; the remaining three were superficial BCE, morphea type of BCE, and the fibroepithelioma type of BCE (Pinkus). There were nine males, three females, varying in age from 23-65.

2) Results of treatment are as follows:

BCNU 0.1%	26 lesions	10 cures	15 partial responses	one no response
CCNU 1.0%	7 lesions	1 cure	5 partial responses	one no response

Partial response is defined as visible flattening of the tumor mass. The ten BCNU cures were in four patients.

3) To date, neither recurrences nor signs of chronic toxicity have been observed.

4) Severe contact dermatitis has occurred in some patients with 0.1% BCNU ointment. The dermatitis appeared from day 2 to day 33 in the different patients. Several patients have not developed any cutaneous irritation. In three patients tumors disappeared without antecedent skin inflammation. Usually the patients who had no skin reaction to the medication had little to no therapeutic response. This supports the clinical impression derived from experience with topical 5-FU: No inflammation, no response.

5) The main difficulty in conducting this study was the lack of an assay system for the stability of the ointment. The active drugs are unstable in water or propylene glycol, therefore, petrolatum is the only suitable vehicle, and it is not possible to extract the active drug from the petrolatum ointment. This made establishing of the shelf-life difficult. By experience, we determined that ointments over one month were not stable.

Significance to Cancer Research:

1) This is an exploratory study of a new drug in skin cancer prompted by a desire to find a superior topical agent to 5-Fluorouracil in propylene glycol which is in widespread use.

2) We would also hope to contribute to the knowledge of the clinical

pharmacology of this agent. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which would allow for long term studies.

Proposed Course of Project:

Concluded except for continued observation of test subjects to determine recurrence rate and for any signs of chronic toxicity.

Keyword Descriptors:

Skin Cancer, Topical BCNU and CCNU, Therapeutic Effectiveness and Toxicity, Contact Dermatitis, Pharmaceutical Stability.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 3632-05 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Toxic Epidermal Necrolysis and Graft-Versus-Host Reaction

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: P.M. Elias, M.D., G.P. Herzig, M.D., R.G. Graw, Jr., M.D.

Cooperating Units: Hematology Supportive Care Branch, NCI

Man Years: (July 1, 1974 through June 30, 1975)

Total:	.01
Professional:	.01
Others:	0

Project Description:

Objectives:

To study Graft-Versus-Host Reaction (GVH) and Toxic Epidermal Necrolysis (TEN), and to investigate the relationship between them.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Methods Employed:

1) Taking of history, and repeated physical examination of a patient with acute lymphocytic leukemia, treated with allogeneic bone marrow transplantation, who developed GVH, and subsequently, TEN.

2) Review of literature

3) Examination with the electron microscope of multiple skin biopsies taken at various time intervals and at various skin sites.

Major Findings:

1) This is the first documented case in which the usual morbilliform eruption associated with sheet-like desquamation of the epidermis,

typical of TEN.

2) Early in the clinical course, cutaneous histopathology was typical for GVH.

3) Later in the clinical course, biopsies taken from intensely erythematous sites, near areas of massive epidermal desquamation showed more extensive epidermal destruction, consistent with the diagnosis of TEN.

4) Support for our thesis linking TEN in this patient with his GVH comes from animal studies in which experimental GVH in hamsters and monkeys developed TEN. Furthermore, a case of GVH was found in the literature in which a bullous eruption, in retrospect compatible with TEN, developed.

5) Electron microscopic findings: In biopsies taken at distances greater than 10 cm. from the blister edge, ultrastructural changes were limited to the basal cell layer. The entire basal layer was involved in a uniform manner, while the spinous, granular, and horny layers appear virtually uninvolved. Mitochondrial swelling, coarsening of tonofibrils and increased prominence of ribosomes were noted in all basal cells. The nuclei appeared contracted, with more convoluted nuclear membranes; increased density of chromatin was noted. Biopsies closer to the blister edge displayed considerable intercellular edema and occasional early bulla formation. Lymphocytes were occasionally seen within the basal layer, but only rarely above. Increased numbers of Odland bodies and abnormal keratohyalin granules were noted in the superficial epidermis. In summary, the initial pathologic events are in the basal layer, and it appears that the mononuclear infiltrate may play a significant pathogenetic role.

Significance to Cancer Research:

1) Immunosuppressed patients, including treated cancer patients, who are transfused or transplanted with immunocompetent cells, may expect to develop GVH. In view of this case report, we may now expect some of them to develop TEN as well.

Proposed Course of Project:

Electron microscopic examination is continuing. Specimens from new patients with either TEN or GVH are also being studied.

Keyword Descriptors:

Topic Epidermal Necrolysis, Graft-Versus-Host Reaction, Electron Microscopic Examination.

Honors and Awards:

First Prize - 1971 North American Clinical Dermatological Society

Memorial Essay Contest

Publications:

1) Peck, G.L., Herzig, G.P., and Elias, P.M.: Toxic epidermal necrolysis in a patient with graft-versus-host reaction. Arch. Derm. 105; 561-569, 1972.

2) Peck, G.L., Elias, P.M., Graw, Jr., R.G.: Graft-versus-host reaction and toxic epidermal necrolysis. Lancet II (7792): 1151-1153 1972.

3) Elias, P.M., Arndt, K.A., Peck, G.L.: Scalded skin syndrome in adults. New Eng. J. Med. 288: 582-583, 1973.

Project No. Z01 CBD 3633-04 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Treatment of Psoriasis with Topical CCNU. (IND-7003)

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Stephen Guss, M.D., and Douglas Key, M.D.

Cooperating Units: None

Man Years (July 1, 1974 through June 30, 1975)

Total:	.01
Professional:	.01
Other:	0

Project Description:

Objectives:

To evaluate safety and effectiveness of topical CCNU in psoriatic patients.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Agents:

0.4% and 1.0% CCNU in 30% liquid petrolatum and 70% white petrolatum.

Material:

Adult patients with psoriasis. Females must be past the childbearing age.

Methods Employed:

1) Patients must have psoriasis previously unresponsive to standard therapy.
2) They must have normal kidney, liver, and bone marrow function.
3) Medication will be applied twice daily until clear.

4) Patients will avoid sun exposure to prevent phototoxicity, if any.
5) Laboratory tests measuring bone marrow, liver, and kidney function will be obtained before, during, and 6 weeks after cessation of therapy.
6) Serial photographs will be obtained.

Major Findings:

1) Seven of 9 patients treated for longer than 2 weeks cleared completely. The 2 patients who did not clear had severe psoriasis of the palms and soles. Four additional patients were treated for less than 2 weeks.
2) Severe irritation of the skin surrounding the psoriatic plaques occurred. This was especially marked in the intertrigenous areas.
3) Subsequent to the cutaneous irritation, persistent pigmentation, telangiectasia, and cutaneous pain developed as well as temporary bone marrow suppression.
4) Two patients have remained free of disease in CCNU treated areas since October, 1970, to the present.
5) No signs of delayed or chronic toxicity have appeared.

Significance to Cancer Research:

1) This study is part of an ongoing search for an effective topical therapy for psoriasis.
2) It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.

Proposed Course of Project:

Concluded except for continued observation of test subjects - for any chronic toxicity and for determination of length of remission.

Keyword Descriptors:

Evaluate safety and effectiveness, temporary bone marrow suppression, prolonged remissions.

Honors and Awards: None

Publications:

1) Peck, G.L., Guss, S.B., and Key, D.J.: Topical Lomustine in the Treatment of Psoriasis. Arch. Derm. 106: 172-176, 1972.

2) Peck, G.L., Guss, S.B., and Key, D.J.: Reply to Comment on Topical Lomustine Therapy for Psoriasis. Arch. Derm. 108:273-274, 1973.

Project No. Z01 CBD 3638-06 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of DNA repair and UV-associated processes in normal human cells and in cells from patients with xeroderma pigmentosum

Previous Serial Number: Same

Principal Investigator: Jay H. Robbins, M.D.

Other Investigators: Alan D. Andrews, M.D., Evelyn Parker, Ph.D.

Cooperating Units: Rufus S. Day, III, Ph.D., Chemistry Branch, DECP, NCI; Steven B. Zimmerman, Ph.D., Laboratory of Molecular Biology, NIAMD

Man Years (July 1, 1974 through June 30, 1975)

Total:	4.5
Professional:	2.5
Other:	2.0

Project Description:

Objectives:

To study the DNA excisional repair process in normal cells and in cells from patients with xeroderma pigmentosum (XP). The role of this process in repair of damage caused by ultraviolet (UV) radiation, alkylating agents and other chemicals is being evaluated. Long-term lymphoblastoid lines derived from XP patients' peripheral blood are being established in culture. The relationship between abnormalities in the function of DNA repair and the development of skin and other tumors in humans is being investigated.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Materials:

Cells are obtained from hospitalized patients and outpatients. Cells studied include: XP and normal peripheral blood lymphocytes, dermal fibroblasts, and long-term lymphoid cell lines derived from peripheral blood cells.

Methods Employed:

Cells used are cultured by standard tissue culture methods. Fibroblast heterokaryons are formed by virus-induced fusion of cells from pairs of XP patients. The cells are cultured and exposed to UV light and then incubated with tritiated thymidine. DNA repair is assayed by measuring the UV-induced unscheduled thymidine incorporation by autoradiography and scintillation spectroscopy.

Major Findings:

1) Genetic complementation studies are being performed in vitro in heterokaryons formed by fusing fibroblasts from pairs of XP patients. Thus, by combining two different XP cells it is possible to ascertain whether they have sufficiently different genotypes to permit restoration of the DNA repair process in the nuclei of the multinucleated heterokaryons. Two cell strains whose nuclei fail to show such restoration of the DNA repair process are said to be in the same complementation group and can be expected to have a genetic lesion affecting the identical gene product. Thus far, four complementation groups (designated A-D) have been found among the patients of the NIH XP series, indicating the existence of at least four loci at which mutations can produce the XP repair defect. The four complementation groups were found to correlate with the magnitude of the repair defect as reflected in the rate of UV-induced thymidine incorporation. Neurological abnormalities attributable to XP have been found only in patients of groups A and D. The neurological abnormalities of XP may therefore be caused only by the specific mutations associated with these complementation groups.

2) We have extended our complementation analysis of various lines of XP cells in collaboration with the laboratory of Prof. Dirk Bootsma, Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands. Prof. Bootsma's group had reported finding three complementation groups among their XP cell lines. Studies in each of our laboratories have found that two of our groups are different from any of their groups. Thus, there appear to be at least five different XP complementation groups, suggesting that defects at any one of at least five genetic loci can give rise to XP. The results of this collaborative study are being prepared for publication.

3) Studies are being conducted in cooperation with Dr. Rufus Day III of the National Cancer Institute, to determine whether complementing fibroblasts restore biological function to UV-damaged DNA. We used the host-cell reactivating system of Day in which fibroblasts capable of repair restore biological function to UV-irradiated adenovirus 2. It was found that within fused populations of XP cells belonging to two different complementation groups there is a sub-population of cells capable of repairing the irradiated virus as well as normal cells.

4) We have been interested in the possible relationship between ageing and the ability to repair damage to DNA. In order to determine the effect, if any, of the number of cell divisions undergone in culture on the ability of cells to repair UV-induced damage to DNA, we are currently comparing rates of UV-induced DNA repair in the same cell line at different passage numbers for cells from most of our XP patients, some of their parents (heterozygotes), and normal donors. These cell lines have been carried through numerous cell passages for use by the American Type Culture Collection. Studies are currently underway to determine the effect, if any, of the cell passage number on the cells' rate of UV-induced DNA repair. We hope these studies will also cast some light on discrepancies between results reported in different laboratories using the same cell line at different passage numbers.

5) We have established long-term lymphoblastoid lines from XP patients' peripheral blood. The existence of such long-term cultures makes possible several experiments not easily performed with fibroblast strains and also makes it possible to obtain large quantities of XP cells for biochemical studies designed to characterize and purify the defective DNA repair enzymes in XP cells. The long-term XP lymphoid lines are free of mycroplasma, have decreased DNA repair, and are sensitive to the killing effects of UV radiation. While these lines have so far been derived only from patients in complementation group C, experiments are underway to establish from patients in all the complementations groups.

6) Studies are being performed with Dr. Steven Zimmerman to determine the levels of DNA ligase activity in fibroblasts from XP variants and from XP patients with decreased UV-induced DNA repair rates. Results to date indicate that these cells have normal levels of ligase activity.

7) Studies are being conducted to determine the effect of different culture conditions on the rate of UV-induced unscheduled thymidine incorporation into fibroblasts. It has been found that such incorporation is markedly influenced by differences in the concentration of fetal calf serum in the culture medium.

8) Studies are being performed to determine the sensitivity of fibroblast strains to ultraviolet radiation. Results of such "post-UV colony forming ability" studies show that XP repair strains within a complementation group have similar degrees of sensitivity to UV radiation. Furthermore, we have found that the different groups can have different sensitivities to UV radiation. Strains in groups A and D are most sensitive, while strains in groups C are less sensitive. The XP variant strain, XP4BE, has been found to have a small but significant increased sensitivity to UV radiation in comparison to normal fibroblasts.

Significance to Biomedical Research and the Program of the Institute:

UV-radiation is believed to be the major cause of the most common type of human cancer, cancer of the skin. Patients with XP are particularly susceptible to the carcinogenic action of UV-radiation and develop multiple malignancies on sun-exposed areas of skin. Normal human cells have an excisional repair process which rapidly repairs DNA damage while most XP patients have a marked impairment in the rate of such repair. This process is involved not only in repair of UV-induced DNA damage but also in repair of damage by certain chemical carcinogens. Understanding the relationship between DNA repair deficiency and skin tumor development in XP patients would, therefore, elucidate the role of DNA repair in preventing in other humans those cancers which may be due to certain chemical and physical carcinogens.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Keyword Descriptors:

Xeroderma pigmentosum, DNA repair, ultraviolet carcinogenesis, mutations, somatic cell genetics, UV-sensitivity, skin cancers, genetic complementation, genetic disease, lymphoblastoid lines.

Honors and Awards:

None

Publications:

1) Andrews, A.D., Robbins, J.H., Kraemer, K.H., and Buell, D.N.: Xeroderma pigmentosum long-term lymphoid lines with increased ultraviolet sensitivity. J. Natl. Cancer Inst. 53:691-693, 1974.

2) Kraemer, K.H., Coon, H.G., Petinga, R.A., Barrett, S.F., Rahe, A.E., and Robbins, J.H.: Genetic heterogeneity in xeroderma pigmentosum: Complementation groups and their relationship to DNA repair rates. Proc. Nat. Acad. Sci. USA 72:59-63, 1975.

3) Robbins, J.H., Kraemer, K.H., and Flaxman, B.A.: DNA repair in tumor cells from the variant form of xeroderma pigmentosum. J. Invest. Dermatol. 64:150-155, 1975.

Project No. Z01 CBD 3643-05 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Wound Healing, Dermatome Stripping, and Dermabrasion Therapy in Darier's Disease (Keratosis Follicularis)

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: L. Dellon, M.D., P. Chretien, M.D., I. Cohen, M.D., W.G. Klingler, M.D., K. Kraemer, M.D., P. Elias, M.D.

Cooperating Units: Surgery Branch, NCI

Man Years: (July 1, 1974 through June 30, 1975)

Total:	.15
Professional:	.15
Others:	0

Project Description:

Objectives:

1) To observe the response of Darier's Disease to surgical therapy.

2) To study the effect of experimental wounding on the clinical course of both involved and uninvolved skin.

3) To determine if the accelerated healing of dermatome stripped diseases skin in Darier's disease is due to an accelerated epidermal migration.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

Normal and diseased skin in a patient with Darier's Disease.

Methods Employed:

1) Dermatome Stripping in patient 1. Strips of skin were taken from

a 62-year-old female patient with Darier's disease using a Castroviejo opthalmologic keratome (Storz Instrument Co.). Strips were approximately 2 cm. in width, 5 cm. in length and 0.2 mm. thick, determined by using a 0.2 mm. spacer in the shaving head. Sites included several areas of the back, upper abdomen and hips. Bleeding was controlled with pressure only. Telfa gauze dressings were used for one to two days, and no other topical therapy was applied. The wounds were then left open, measured and photographed daily.

Incisional Wound Healing. Multiple linear incisions, approximately one cm. in length, were made in diseased skin areas of the right anterior forearm and in clinically uninvolved skin of the left anterior forearm. Because the involved skin surface is more irregular than uninvolved skin, and because the epidermis is variably thickened in dermatoses such as Darier's disease, the standard blade guard device cannot be used to ensure equal depth of incisions. We, therefore, chose minimal capillary bleeding as a measure of comparability of incision depth. Telfa gauze was the only dressing employed. Four mm. punch biopsies were taken at zero time, and 3, 6, 12, 18, 24, 48, and 72 hours after wounding. Each biopsy was divided transversely to the wound into two approximately equal pieces. One piece was fixed in cold phosphate-buffered glutaraldehyde, post-fixed in osmium tetroxide, imbedded in Epon 812, and 1-2 u thick sections were stained with Azure II-methylene blue. The other half was routinely fixed in formaldehyde and imbedded in paraffin prior to sectioning and staining. Sections were cut coronally to the incision.

2) Dermabrasion Therapy in patient 2. A 26-year-old man with hypertrophic conifying Darier's disease especially severe on arms and legs was treated with surgical dermabrasion under general anesthesia several times. Rate of wound healing was measured by serial 6 mm punch biopsies of circular defects produced by 3 mm punches, and by serial punch biopsies taken from dermatome stripped sites of diseased and uninvolved skin punches.

3) Dermatome Stripping in patients 3 to 5. Two women and one man with severe, confluent, inflammatory, vesicular Darier's disease was treated with dermatome stripping of legs or buttocks under general anesthesia. Rate of wound healing was measured by the methods used in patient 2. One of these women also had dermabrasion of the face and excision of severely diseased labia majora.

Major Findings:

1) Dermatome Stripping in patient 1. Involved sites healed more rapidly than uninvolved skin following dermatome stripping. Two weeks after stripping, the uninvolved site remainded larger and more erythematous than the involved site. Furthermore, a zone approximately 10 cm. wide of previously diseased skin around stripped sites on the left hip was observed to have clinically regressed by this time. Clinical regression of involved skin surrounding dermatome stripped sites was repeatedly observed.

Five months after the initial stripping experiment, the patient had a generalized exacerbation of her disease, which spared previously dermatomed sites of uninvolved skin. Clinically, the uninvolved stripped sites are now hypopigmented and slightly atrophic. Histologically, the epidermis and pilosebaceous apparatus appear normal except for diminution of the rete ridge pattern and flattening of the papillary dermis.

Incisional Wound Healing. Superficial linear incisions healed more quickly in diseased sites (12 hours) than in uninvolved sites (48 hours) indicating accelerated epidermal migration.

2) Diseases sites healed more rapidly than uninvolved sites, using both methods of measuring rate of wound healing. Incisional wound healing studies were not done because of the difficulty in locating incisions in diseased tissue after 1-2 days. 3 mm defects in diseased skin healed in 2 d, and in uninvolved skin in 3 d. The patient responded well to dermabrasion and had less than 10% recurrence after 11 months of observation. Peripheral clearing, as seen in patient 1, was not observed.

3) Dermatome stripping was well tolerated in all patients producing long lasting (over 3 years) disease free remissions. Peripheral clearing has occurred in 3 of 5 patients following dermatome stripping.

Significance to Cancer Research:

1) Knowledge of the controls governing cell migration after injury are essential to the understanding of malignant growth.

Proposed Course of Project:

1) Ultrastructural examination of the above material.

2) Confirmation of these findings in other patients with Darier's disease.

Keyword Descriptors:

Accelerated Wound Healing, Dermatome Stripping, Dermabrasion Therapy, Darier's Disease.

Honors and Awards: None

Publications:

Peck, G.L., and Elias, P.M.: Wound Healing in a Patient with Darier's Disease: Preliminary Observations. In Maibach, H.I. and Rovee, D. (Eds.): Epidermal Wound Healing. Chicago, Ill., Year Book Medical Publishers, 1972, pp. 357-363.

Dellon, A.L., Peck, G.L., Chretien, P.B.: Hypopharyngeal and laryngeal involvement with Darier's Disease. Arch. Dermatol. (in press) (approx. May '75)

Peck, G.L., Kraemer, K.H., Wetzel, B., Klingler, W.G., Cohen, I.K.: Cornifying Darier's Disease - A unique variant. I-Case Report Arch. Dermatol. (in press).

Cohen, I.K., Kraemer, K.H., Peck, G.L.: Cornifying Darier's Disease - A unique variant. II. Surgical Treatment. Arch. Dermatol. (in press).

Project No. Z01 CBD 3644-04 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Establishment of a Screening System for Topical Chemotherapeutic Agents in the Therapy of Skin Cancer, Mycosis Fungoides, and Psoriasis

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Stephen K. Carter, M.D.

Cooperating Units: Division of Cancer Treatment, Cancer Therapy Evaluation

Man Years (July 1, 1974 through June 30, 1975)

Total:	.10
Professional:	.10
Other:	0

Project Description:

Objectives:

The establishment of a scientific program in which present and future chemotherapeutic agents may be screened topically for antimitotic potential, toxicity, and possible therapeutic use initially in malignant skin diseases, such as cutaneous carcinoma and mycosis fungoides, and subsequently in benign hyperplastic diseases such as psoriasis.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.2: Discover new therapeutic agents and develop means for their selection.

Patients Needed:

At least 10 adult men or women for each chemotherapeutic agent tested topically in the treatment of skin cancer, mycosis fungoides or psoriasis. Females must be past the childbearing age.

Methods Employed:

After chemotherapeutic agents have been supplied by the Division of Cancer Treatment, NCI, and after these agents have been screened for biological activity and safety in experimental models, then large scale clinical testing will be done in mycosis fungoides, psoriasis, and skin cancer. It is anticipated that the initial steps in this project will be patch test screening for biological activity in patients with psoriasis and mycosis fungoides and percutaneous absorption studies with radiolabelled chemotherapeutic agents in laboratory animals.

The clinical protocols of this large scale clinical testing have not been formulated as yet. However, the basic design will be similar to NCI-3631 and NCI-3633.

Major Findings:

In progress.

Significance to Cancer Research:

1) This program is a national cooperative study designed to discover superior topical chemotherapeutic agents than those which are currently available in the treatment of skin cancer, mycosis fungoides, and psoriasis.
2) We would also hope to contribute to the knowledge of the clinical pharmacology of these agents. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which allows for long term studies.

Proposed Course of Project:

The entire design of this screening system, including preclinical screens and large scale clinical testing, is currently being established with the assistance of a committee of dermatologists, pharmacologists, toxicologists, and chemotherapists. This program will complement an Arthritis Institute program for psoriasis.

Keyword Descriptors:

Topical chemotherapy, skin cancer, Mycosis Fungoides, Psoriasis, national cooperative study, and preclinical screens.

Honors and Awards: None

Publications: None

Project No. Z01 CBD 3647-02 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The fine surface morphology, ultrastructure, and cytochemistry of normal and diseased leukocytes during development in the circulation, and in sites of leukocytic infiltration.

Previous Serial Number: None

Principal Investigator: Bruce Wetzel, Ph.D.

Other Investigators: Elaine B. Alexander, M.D., Sharon Wahl, Ph.D., and Leonard Altman, M.D.

Cooperating Units: Immunology Branch, NCI

Man Years (July 1, 1974 through June 30, 1975)

Total:	2.25
Professional:	0.25
Other:	

Project Description:

Objectives:

To define the surface ultrastructure of leukocytes under a wide range of conditions and thereby provide fresh insight into the physiology of normal and leukemic cells; also, to characterize definitively the origin, contents, and fate of the characteristic granules of neutrophils, eosinophils, and basophils as an index of the development, function and fate of these cells.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

Aliquots of white cells, concentrated from the anticoagulated blood of normal human donors as buffy coats and by the Ficoll-Hypaque procedure, were fixed in suspension for SEM, and parallel aliquots were incubated on nylon fiber columns; non-adherent and adherent leukocyte populations were collected from the columns and fixed in suspension for scanning electron microscopy (SEM). Leukocytes fixed in suspension were deposited, stained, and washed on Nucleopore filters, trapped in a thin film of water, quick frozen, and freeze-dried for SEM with 90% yields. Individual cells examined by SEM were relocated by light microscopy (LM) and additional specimens have been prepared for transmission electron microscopy (TEM).

Chemotactic studies were carried out with guinea pig peritoneal macrophages in modified Boyden chambers equipped with Nuclepore filters (3.0 um pore size). The response of leukocytes on one side of the filter to the presence of control phosphate-buffered salt solution (PBS) or to the chemotactic factors C5A and LDCF on the other side was studied by fixing the filters at different times (30, 60, 90, and 120 minutes). Light microscopy was done on some of the same scanned cells as above.

Major Findings:

Normal human peripheral lymphocytes almost invariably appear villous when fixed in suspension by our standard procedures. Lymphocytes fixed directly in conventional buffy coats, in Ficoll-Hypaque bands, and in subsequent washes appear as a single population of moderately villous cells with little variation in form. Also, when fixed in suspension non-adherent cells (90% "T", lacking surface immunoglobulin) and adherent cells (60% of lymphocytes "B" cells with surface immunoglobulin) from the nylon column have the same villous surface and are indistinguishable from each other. Rapid and substantial changes in lymphocyte surface features occur as the cells contact certain surfaces, notably the silver filters widely used to collect these cells for SEM. These changes can account for the various forms reported by others as distinctive "B" and "T" cell topography.

During the chemotactic assay procedure, guinea pig macrophages display kaleidoscopic changes from their ridged spherical form in the washed cell suspension. These changes are specific and reproducible. In the absence of chemotactic factor on the far side of the filter, relatively few cells adhere by 30', few cells spread or appear to be moving by 60' and the cells show little ruffling and rarely migrate through the pores. C5A induces rapid adhesion, spreading, and motility directed through the pores as early as 30'; by 60' many cells reach the distal surface and most are remarkably flat and smooth; by 90' their shape diversifies and prominent localized ruffles appear on most cells of all shapes, ie., flattened, spherical, stellate, and fusiform. LDCF produces a somewhat different series of changes and most cells cross the filter by 60'. By 60' with LDCF and by 120' with C5A and PBS most cells are either stellate with very long processes and deeply ridged surfaces or spherical with blebbed or smooth surfaces. These changes represent a dynamic response to a positive chemotactic gradient by adhesion, locomotion, spreading, and transient ruffling. By LM, macrophages (80% of the population) were identified in all of the forms noted above (except spherical smooth cells which all contained pycnotic nuclei).

Significance to Cancer Research:

The reactions of lymphocytes and macrophages to immune responses in malignant growth and therapy are largely dependent on cell surface phenomena and cell interaction -- both uniquely amenable to study by SEM and ancillary methods.

A thorough understanding of the origin and nature of leukocyte granules is necessary to define and interpret the differentiation of normal granulocytes, the cytopathology of chronic granulocytic leukemia and related disorders, and to help clarify the nature and functions of granulocytic infiltrates in skin and other tissues in malignant diseases.

Proposed Course of Project:

We are engaged in a thorough description of the topography of peripheral leukocytes from normal humans obtained in representative yields. We are exploring new methods for simultaneous identification of these cells within the SEM. This approach will be extended to studies of hematopoiesis and the leukemias. A major study of leukocyte granulogenesis is planned employing quantitative autoradiography, cytochemistry and TEM. Light microscopic monitoring of preparative conditions and the dynamics of leukocyte adherence, motility, interaction, and growth will be continued with the use of thin perfusion chambers.

Keyword Descriptors:

Leukocytes, cell surface, lymphocytes, macrophages, chemotaxis, SEM, B cells, T cells, human peripheral blood, nylon column cell separation, buffy coat, Ficoll-Hypaque separation.

Honors and Awards: None

Publications:

1) Alexander, E., and Wetzel, B.: Human lymphocytes -- similarity of B and T cell surface morphology. Science (in press).

Project No. Z01 CBD 3648-03 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: T Cell Leukemias and Lymphomas: Morphologic and Functional Studies of the Neoplastic Cells

Previous Serial Numbers: NCI-3648 and NCI-3653

Principal Investigator: Marvin A. Lutzner, M.D.

Other Investigators: Richard L. Edelson, M.D., Allan D. Andrews, M.D.

Cooperating Units: Philip Schein, M.D., Medicine Branch, NCI, Ira Green, M.D., and Charles Kirkpatrick, M.D., National Institute of Allergy and Infectious Disease; Tab Ahmed and Michael Facktor, U.S. Naval Hospital.

Man Years (July 1, 1974 through June 30, 1975)

Total:	2.0
Professional:	2.0
Other:	0

Project Description:

Objectives

1) To determine whether those neoplastic cells which preferentially infiltrate the skin share common features.

2) To relate the clinical findings in these patients to the distinctive properties of the neoplastic cells.

3) To establish more effective means of therapy of these disorders through exploitation of the distinctive properties of the neoplastic cells.

4) To use the homogenous cells found in these disorders as reagents for investigation of T cell function.

5) To isolate the product of the mixed leukocyte reaction locus.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

1) Tissues to be studied are sectioned in a cryostat, layered with sheep erythrocytes containing on their surfaces moieties which will specifically interact with receptors on mononuclear cells, rinsed to remove nonadherent cells, fixed in glutaraldehyde, and stained with H and E to be viewed by light microscopy.

2) Tissue, cells and buffy coat preparations are Feulgen-stained and examined with cytophotometer in order to detect presence of polyploid cells.

3) Lymphocytes are grown in short term culture and the karyotype determined.

4) Lymphocytes and tissue were fixed and stained routinely for electron microscopy.

5) Lymphocytes are tested for responsiveness to the mitogens phytohemagglutinin, pokeweed and concanavalin A and for stimulating and responding capabilities in mixed leukocyte cultures.

6) Lymphocytes are tested for sheep erythrocyte receptors, complement receptors, Fc receptors, IgG receptors, surface immunoglobulin, and specific antigenic determinants.

7) Lymphocytes, fibroblasts and keratinocytes are tested for effector and target cell potential in cell mediated lympholysis reaction.

8) An antiserum specific for T cells has been produced and purified by absorption with B cells, red cells, and platelets. This antiserum may be used therapeutically in cases unresponsive to other more conservative measures.

9) Leukapheresis, a process by which blood is passed through a continuous flow centrifuge with white cells being selectively removed from the buffy coat, has been used in the therapy of one patient.

Major Findings:

1) T cells, of at least certain morphologic varieties, preferentially infiltrate the skin and spare the bone marrow.

2) If the Sezary syndrome is redefined as T-cell leukemia with associated erythroderma, we have now identified three morphologic variants.

3) Each patient is this expanded Sezary group differs from the other patients at the cellular level, using functional, antigenic and morphologic criteria. In addition, several patients had unique clinical features. i.e. Nathan's inverse cyclicity and Stern's recurrent hepatic and renal infiltration.

4) The first case of lymphocytic leukemia with both B and T cell markers on the same cells was identified.

5) Review of the cumulative NIH experience with the Sezary syndrome revealed a remarkable lack of thymic infiltration at autopsy. This plus the identification of T cells in the skin of our more recent patients suggests that the skin may actually be playing a role in the aberrant differentiation of these abnormal cells.

6) An affinity of T cells for the skin is supported by our observation that the large majority of cells in mycosis fungoides, lichen planus, Mucha Habermann's disease, parapsoriasis en plaque and lymphomatoid papulosis also appear to be T cells.

7) One patient with T cell leukemia and tumor stage mycosis fungoides superimposed on a generalized erythroderma was treated with leukapheresis, a process by which leukocytes are selectively removed from the buffy coat in a continuous flow centrifuge, and the cutaneous lesions resolved. The patient returned 100 days later from California for a repeat of the leukapheresis with similar results.

8) Cells from one patient with the Sezary syndrome have been maintained in culture for one month and retain both their receptors for sheep erythrocytes and their distinctive morphology.

9) An immunotherapy protocol has been devised and accepted for use. However, because of the relative success of our more conservative therapeutic approaches, immunotherapy has not yet been required. Other approaches include the present trial run of L-asparaginase which appears to be of at least limited efficacy.

10) Three patients with cutaneous T cell leukemia have now been treated by leukapheresis. One patient, who presented at the time of initial therapy, with tumor stage mycosis fungoides superimposed on a generalized erythroderma and with a lymphocytosis of more than 200,000 per cu mm has been adequately managed for the last 18 months by leukapheresis alone. Two other patients have also significantly benefited from leukapheresis. One patient is presently undergoing extracorporeal irradiation to determine whether this is a more efficient means of managing patients with lower peripheral blood lymphocyte counts.

11) Cells from one patient were hypotonically lysed following leukapheresis and membrane components were conjugated to a cyanogen bromide activated sephadex column. This column was subsequently used as an immuno-absorbent column to remove and then concentrate the anti-T cell activity and the active factors are themselves valuable reagents.

12) Serum from each of 6 tested patients with cutaneous T cell leukemia contained large amounts of macrophage inhibitory factor, which could be traced back to the leukemia cells. The serum of these patients therefore

represent reservoirs from which this material can be isolated and characterized. Of clinical significance, the presence in the serum of a factor which has a profound effect on circulating monocytes may be at least in part responsible for observed anergy in the patients and their susceptibility to infection.

13) Patients with lymphomatoid papulosis were evaluated with regard to their clinical presentation, microscopic, ultrastructural and cell surface marker characteristics of their cutaneous infiltrates, their response to challenge with intradermal antigens, and the response of their peripheral leukocytes to mitogen and antigen stimulation.

Preliminary findings revealed that the infiltrating cells of lymphomatoid papulosis were thymus-derived lymphocytes and the nuclear irregularity of the cell of the cutaneous infiltrates was significantly greater than that of infiltrating lymphocytes of delayed hypersensitivity skin reactions, with a pattern similar to that seen in the circulating lymphocytes of the Sezary syndrome.

Patients with lymphomatoid papulosis demonstrated normal reactivity to intradermally applied antigen and normal in vitro stimulation of lymphocytes by mitogens.

Significance to Cancer Research:

By identifying the roles of mononuclear cells in defense against non-lymphoid cancer and accurately characterizing the types of lymphoid cells causing various lymphomas, a more rational approach to the treatment and prevention of these diseases is possible.

Proposed Course of Project:

We hope to characterize a broad spectrum of cutaneous disease so that the underlying pathologic processes will be better understood. This work will involve the study both of patients and experimental diseases.

Keyword Descriptors:

Lymphocytes, lymphoma, leukemia, Sezary syndrome, mycosis fungoides

Honors and Awards: None

Publications:

1) Edelson, R., Smith, R., Frank, M., and Green, I.: Identification of subpopulations of mononuclear cells in cutaneous infiltrates. J. Invest. Dermatol. 61: 82-89, 1973.

2) Shevach, E.M., Edelson, R.L., Frank, M., Lutzner, M.A., and Green I.: A human leukemia cell with both B and T cell surface receptors. Proc. Natl. Acad. Sci. 71: 863-866, 1974.

3) Smith, R.W., Blaese, R.M., Hathcock, K.S., Buell, D.N., Edelson, R.L., and Lutzner, M.A.: T and B lymphocyte markers in lymphoid cell research and in human diseases. Eighth Leukocyte Culture Conference (in press).

4) Edelson, R.L., Kiekpatrick, C.H., Shevach, E.M., Schein, P.S., Smith, R.W., Green, I., and Lutzner, M.A.: Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes: Morphologic and functional studies; Ann. Inter. Med. (in press) June, 1974.

5) Lutzner, M.A., Edelson, R.L., and Schein, P.: Sezary syndrome (chapter) in Current Dermatologic Management. (in press).

6) Yoshida, T., Edelson, R., Cohen, S., and Green, I.: Serum macrophage inhibitory factor in patients with T cell leukemia, J. Immunol (in press).

7) Gerna-Torsellini, M., Edelson, R.L., Frank, M.M., and Kaplan, A.: Identification of subpopulations of mononuclear cells in cutaneous infiltrates, II. Urticaria. Bolletino Sierroterapica Milanese (in press).

8) Ahmed, A., Edelson, R.L., Strong, D., and Green, I.: In vitro reactivity of lymphocytes from patients with chronic T cell leukemia. Clin Exp Immunol (in press).

9) Green, I., Jaffe, E., Shevach, E., Edelson, R., Frank, M., Berard, C.: Determination of the origin of malignant reticular cells by the use of surface markers. Monograph - International Academy of Pathology (in press).

Project No. Z01 CBD 3640-03 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Enzymatic Control Mechanisms in Melanogenesis

Previous Serial Number: Same

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total:	1.6
Professional:	.6
Others:	1.0

Project Description:

Objectives:

To investigate the control mechanisms involved in regulating melanogenesis in a variety of normal and malignant tissues.

The objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

Tyrosinase and other enzymic activities are investigated both by spectrophotometric and radioactive assay methodology. Tissues are dissected immediately before use in 0.1 M phosphate buffer, homogenized and fractionated into subcellular fractions by means of density gradient centrifugation and differential centrifugation. After solubilization with detergents, enzymes are further purified by polyacrylamide gel electrophoresis. Samples are then incubated with the appropriate substrates and controls. The production of melanin and other reaction products can be followed spectrophotometrically or by liquid scintillation counting of the newly formed radioactive melanin or byproducts.

Major Findings:

Since only one enzyme (tyrosinase) is essential for melanin biosynthesis, it is a unique system for the study of enzymatic control mechanisms in normal and malignant tissues. We have examined the status of enzymatic control of melanogenesis and have found that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary systems, but not peroxidase, as has been suggested by some workers. It has always been a subject of dispute how tyrosinase, which can be easily demonstrated to be present in an active configuration in the endoplasmic reticulum and Golgi apparatus, is inactivated in vivo in mammalian systems and subsequently activated once in situ in the melanosome. In amphibians, the mechanism seems to be proteolytic activation; in mammalian and avian systems, however, proteolysis has no function in this regard. It has been found in this study that tyrosinase is under allosteric control and that phospholipids may play a part in the expression of the enzyme's activity. It has also been found that this allosteric regulation in murine melanomas is altered; this perhaps explains the atypical melanosome formation in these tissues.

Significance to Cancer Research:

In view of the differences noted between the regulation of tyrosinase in normal and melanoma tissues, we feel that at least one of the primary differences between the melanogenic capabilities of these two tissues has been revealed. The fact that these controls are operational at the post-translational level is informative; whether other levels of cellular control mechanisms are affected by carcinogenesis remains to be investigated. It is hoped that further study of the cause of these enzymatic differences in the malignant tissues will provide insights into the nature of neoplastic transformation.

Proposed Course of Project:

Differences in the control mechanisms over melanogenesis in normal and malignant melanoma tissues will continue to be investigated. The characteristics of enzymatic activity and control in various subcellular fractions are being studied. This project has been expanded to include characterization of similar metabolisms in normal and malignant human tissues.

Keyword Descriptors:

Tyrosinase, melanoma, enzyme regulation.

Honors and Awards: None

Publications:

Hearing, V.J.: Mammalian melanogenesis tyrosinase versus peroxidase

involvement, and activation mechanisms. Arch Biochem Biophys 158:720-725, 1973.

Hearing, V.J.: Tyrosinase activity in subcellular fractions of black and albino mice. Nature 245:81-83, 1973.

Hearing, V.J. and Ekel, T.M.: Involvement of tyrosinase in melanin formation in murine melanoma. J. Invest. Derm. 64:80-85 (1975).

Project No. Z01 CBD 3650-03 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Characterization of Mammalian Melanosomes

Previous Serial Number: None

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: Walter G. Klingler, M.D.

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total:	2.4
Professional:	1.4
Others:	1.0

Project Description:

Objectives:

To characterize the structural and enzymatic composition of melanin granules throughout their development in vivo, both in normal and abnormal pigmentary systems.

The objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

Our research has been aimed primarily at characterizing the chemical composition of melanosomes in the mouse, both in the normal black mouse as well as in melanoma tissue. Eyes are excised and homogenized in 0.25M sucrose, and the melanosomes are then isolated and purified by means of an extensive series of differential centrifugations. Granules thus isolated are solubilized in either sodium decyl sulfate, 8 M urea of Triton X-100, then examined with the aid of polyacrylamide gel electrophoresis. Computer analysis of the resulting protein separations allows the calculation of various physio-chemical parameters of these macromolecules, including molecular weight, molecular size, valence and several other physical constants that are most useful in the comparisons of various proteins.

Major Findings:

The melanosome in normal mouse tissue is composed of multiple proteins, many of which are loosely bound and easily extracted. These probably constitute the proteins of the limiting membrane of the organelle. Another protein is of lower molecular weight and is tightly bound to the granule, and probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. Melanosomal proteins from melanoma tissues vary in structure from those of normal tissue. Several of the proteins seem to be missing completely from these granules, which are also structurally distinguishable from normal granules by electron microscopy. Perhaps more importantly, many of the proteins in melanoma melanosomes are unique and are not found in normal melanin granules. This has been found to be the case in human melanoma as well.

Significance to Cancer Research:

The observations concerning the aberrant biochemical characteristics of the melanoma melanosome indicate that the control of melanogenesis in malignant melanoma is in some manner affected by carcinogenesis. Melanomas are unusual among cancers since they do not result in complete differentiation of the affected melanocyte, but allow this cell's specialization, i.e. melanogenesis, to continue. These results indicate that although melanogenesis takes place, its metabolic pattern is abnormal.

There are a wide range of implications of this research for possible immunotherapy and/or immunoassay of human malignant melanosomes. An analogous situation of altered proteins has been described in several other types of malignancies; thus this process of atypical formation of proteins may be common to neoplastic transformation.

Proposed Course of Project:

Since there are indications that the metabolism of melanogenesis in murine melanoma is aberrant, our research is ultimately intended to examine human melanomas, and attempt to characterize the level on which these disorienting control mechanisms operate. It is hoped that further insight into such controls, be they at the level of replication, transcription, translation, or post-translation, will provide clues as to the level on which carcinogenic information is expressed.

Keyword Descriptors:

Melanoma, melanosome, protein synthesis.

Honors and Awards: None

Publications:

Hearing, V.J. and Lutzner, M.A.: Mammalian melanosomes; characterization by polyacrylamide gel electrophoresis, Yale J. Biol. Med., 46:553-559, 1973.

Hearing, V.J. and Eppig, J.J., Jr.: Biochemical aspects of melanogenesis in normal and murine melanoma tissue. J. Cell Biol 59:137a, 1973. (Abstract)

Hearing, V.J. and Eppig, J.J. Jr: Electrophoretic characterization of melanosomal proteins extracted from normal and malignant tissues. Experientia 30, 1011-1012 (1974).

Klingler, W.G., Montague, P.M. and Hearing, V.J.: Unique melanosomal proteins in murine melanoma. Proc IX Inter Conf Pigment Cell Biol (in press).

Klingler, W.G., Montague, P.M. Chretian, P.B., and Hearing, V.J.: Altered melanosomal proteins in human malignant melanoma. Fed. Proc. 34, 872, 1975.

Earle, M. Canellos, G.P. and Hearing, V.J.: Gallium^{67} binding to melanin. Clin. Res. (in press).

Project No. Z01 CBD 3652-02 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Mechanisms of Cellular Immunity in Man

Previous Serial Number: NCI-3652 and NCI-3641

Principal Investigator: William R. Levis, M.D.

Other Investigators: John A. Powell, M.D.

Cooperating Units: None

Man Years (July 1, 1974 through June 30, 1975)

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description:

Objectives:

1) To investigate the mechanisms concerned with the control of mitosis, differentiation, function, and potentialities of normal and pathologic cells, with particular reference to the lymphocyte and cells of the lymphoreticular system.

2) To investigate the functional and morphological interactions between lymphocytes and macrophages and among different populations of lymphocytes during in vitro immune responses related to delayed hypersensitivity, homograft reactions, and cell mediated allergic contact dermatitis.

3) To detect, characterize, and isolate biologically active substances, including soluble mediators of cellular immunity, produced in stimulated and unstimulated human leukocyte cultures. To study their effects on supporting, augmenting, and inhibiting blastogenesis, cell division, modulation, and differentiation in vitro.

4) To develop an in vitro system for studying allergic contact dermatitis in humans including a method of monitoring immunotherapy of skin cancers with DNCB.

5) To develop a useful and sensitive histocompatibility test in humans.

6) To develop an in vitro model for the study of homograft rejection in humans.

7) To develop an in vitro system for studying immune reactivity to various skin cancers.

8) To determine the efficacy of 2,4 dinitrochlorobenzene (DNCB) on cutaneous malignancies and premalignant growth in subjects sensitized to DNCB.

9) To evaluate DNCB as a useful model for the study of immunotherapy of certain human cancers and possibly lead to additional immunotherapy studies.

10) To develop an in vitro model for studying and screening drugs which modulate cell mediated immunity in humans.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (along and in combinations) in the cure and palliation of cancer.

Material:

1) Lymphocytes and other peripheral blood cells are obtained from hospitalized patients and outpatients including patients with chronic lymphocytic leukemia, sezary syndrome, and other disease states.

2) Keratatome strips of human skin.

3) Excised tumors of human skin.

4) Sperm cells obtained from human ejaculates.

Methods Employed:

1) Human peripheral blood lymphocytes are cultured in vitro and stimulated to transform into large blastoid cells by factors such as phytohemagglutinin, antigens, and foreign tissue. This phenomenon of blastogenesis is evaluated by the morphologic and biochemical changes which the lymphocytes undergo. The mechanisms by which these blastogenic factors act and the culture conditions required for blastogenesis are determined.

2) Human peripheral blood leukocytes are separated into a glass-adherent cell population, composed largely of macrophages, and into a population consisting of small lymphocytes. By recombining such populations obtained from the same and from different individuals, the function of each population is determined. Interactions and contacts between cells are examined by light and scanning electron microscopic

techniques, including methods for observing the same cells by both modalities.

3) A method has been developed for freeze storing leukocytes for subsequent studies of the blastogenic response to various antigens and mitogens.

4) A method has been developed for adapting the dinitrochlorobenzene contact sensitization test to the in vitro lymphocyte transformation system.

Major Findings:

1) Methods for obtaining 'purified' lymphocytes, glass-adherent mononuclear cells, and a population containing both cell types from human peripheral blood have been developed and analyzed. We have improved and utilized a very rapid and simple 5-minute centrifugation method using heparinized whole blood for obtaining a population of mononuclear leukocytes (lymphocytes and monocytes) with very few contaminating granulocytes. This mononuclear leukocyte population contains the two cell types required for the blastogenic response and provides cultures capable of responding with maximal responses to specific and nonspecific blastogenic factors.

2) Human epidermal cells induce blastogenesis in allogeneic leukocytes. Human epidermal cells did not induce blastogenesis in autologous leukocytes nor in the leukocytes of a mixed leukocyte HL-A identical sibling pair. These results indicate that epidermal induced leukocyte blastogenesis is a measure of histocompatibility in humans comparable to a one-way mixed leukocyte reaction.

3) A method has been developed which permits positive light microscopic identification of individual leukocytes in a heterogeneous population by Giemsa staining while preserving delicate surface features for subsequent scanning electron microscopy of the same cells.

4) Isolation and partial characterization of a factor released by glass adherent phagocytic cells which is an essential requirement for the blastogenic response of lymphocytes to specific antigens, leukocytes, and phytohemagglutinin.

5) Isolation of a factor released in cultures containing lymphocytes of subjects sensitized to DNCB that will induce blastogenesis in leukocyte cultures from nonsensitized subjects.

6) Characterization of the abnormal blastogenic responses of lymphocytes from patients with chronic lymphocytic leukemia, sezary syndrome, and other related disease states.

7) Development of a method for monitoring the degree of sensitivity to

DNCB in vitro during in vivo contact sensitization. This in vitro technique expands the usefulness of DNCB for the evaluation and study of cell mediated immunity, and offers a method for monitoring DNCB immunotherapy, an approach for the study of tolerance, and a controlled system for the study of the many antigen-released mediators of cellular immunity in man. Similar approaches with other contact allergens may offer a safe diagnostic test for allergic contact dermatitis.

8) Successful adaptation of the Dinitrochlorobenzene (DNCB) contact sensitization test to the in vitro lymphocyte transformation technique in humans.

9) Development of simplified methods for freeze storing human peripheral blood leukocytes.

10) Using the frozen leukocyte technology and the method for studying DNCB sensitivity in vitro we have demonstrated a temporal relationship between in vivo skin reactivity and in vitro specific lymphocyte transformation during a primary immune response.

11) Peak in vitro activity was observed two to four weeks following application of the primary sensitizing dose of DNCB and fell off to lower levels within two months of primary sensitization. These sequential studies of allergic contact dermatitis in humans are the first such studies of the kinetics of a primary cellular immune response using both in vivo and in vitro techniques.

12) Additional studies have demonstrated a quantitative relationship between the intensity and duration of allergic contact dermatitis to DNCB in vivo and the magnitude of specific lymphocyte transformation in vitro.

13) Demonstration that repeated challenge with DNCB boosts specific in vitro lymphocyte transformation.

14) Discovery of lymphokine activity in allergic contact dermatitis there is evidence that such lymphokines may be the mediators of cell mediated immunity.

15) Demonstration of the time course of release of lymphokine activity in human peripheral blood cultures.

16) Demonstration of reproducible dose-response curves for lymphokine activity. Such dose-response curves illustrate a sensitive quantitative assay for cellular immunity in vitro and offers a biological system with great potential for further characterization and isolating the mediators in cell mediated immunity.

17) The DNCB system offered the versatility that made it possible to

show the lymphokines responsible for lymphocyte transformation in target leukocyte populations from nonsensitized subjects were as active on syngeneic (identical twins) leukocytes as on allogeneic leukocytes, indicating the observed activity was not due to histocompatibility factors.

18) It has recently been shown that lymphokine activity is absent during a primary immune response to DNCB and required one or more rechallenges before activity is detectable using the lymphocyte transformation system which suggests the soluble factors known as lymphokines, may represent an amplification of cellular immunity.

19) Successful adaptation of an automated microsystem for harvesting cells had enabled us to harvest cells in a rapid fashion.

20) The unique features of this project are: a) Both in vitro and in vivo portions of this project are ethically feasible in humans which makes these studies more germane to human disease. b) Previous studies with microbial antigens have lacked effective control over exposure, in contrast to controlled DNCB sensitization. c) The assay is sensitive, specific and quantitative making it a valuable tool for measuring lymphocyte activation under a variety of experimental conditions.

21) Studies on soluble factors released by human lymphocytes (lymphokines) indicate that little or no lymphokine is released during an in vivo primary immune response to DNCB, but is produced in some subjects only after additional in vivo rechallenge. We interpret this to indicate that the lymphokine activity demonstrated in vitro may well indicate a bodily mechanism for amplifying the cellular immune response.

22) Recent studies in our laboratory indicate that an entire series of DNP containing amino acids induce varying degrees of lymphocyte transformation specifically in subjects topically sensitized with DNCB. The ability to stimulate with soluble DNP containing substances as well as with our previously developed particulate antigen offers great flexibility in our combines in vitro - in vivo system for investigating the cellular immune response in humans. Furthermore, these studies might prove to be of considerable importance in understanding the mechanism of triggering a specific cell mediated immune reaction.

23) Demonstration of the ability of sperm cells to induce allospecific lymphocyte transformation. We have previously demonstrated the ability of macrophages, certain lymphocytes, and human epidermal cells to induce allospecific lymphocyte transformation. Interestingly, studies in the mouse indicate that a product of the major histocompatibility complex has a tissue distribution similar to the cell populations we have found to induce allospecific lymphocyte transformation in humans. These findings could have important impact in diverse areas such as transplantation and reproductive biology. We are currently attempting to find out if our studies on contact sensitivity are related or in any

way related or controlled by the major histocompatibility complex in humans. The combination of our histocompatibility and contact sensitivity studies may develop into a field of human immunogenetics with potential ramifications in a wide variety of disease states.

Significance to Biomedical Research and the Program of the Institute:

The lymphocyte is a cell involved in immune reactions which may be operative in the immune response to neoplastic tissue. Our studies concerned with blastogenesis and the control of cell division and differentiation of the lymphocyte may elucidate this cell's normal and abnormal functions in the immune response involved in the host's defense against cancer. These studies should also provide information concerning the control of cell differentiation and division.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Keyword Descriptors:

Cell mediated immunity, delayed type hypersensitivity, contact sensitivity allergic contact dermatitis, immunogenetics, lymphokines, dinitrochlorobenzene, sperm, immunotherapy.

Honors and Awards: None

Publications:

1) Miller, A.E., and Levis, W.R.: Lymphocyte transformation during DNCB contact sensitization: An in vitro - in vivo evaluation of the primary immune response in man. J. Clin. Invest. 52: 1925-1930, 1973.

2) Wetzel, B., Erickson, B.W., and Levis, W.R.: The need for positive identification of leukocytes examined by scanning electron microscopy. Scanning Electron Microscopy. Scanning Electron Microscopy. O. Jahri and I. Corwin (Eds.). ITT Research Institute, Chicago, Illinois, 535-542, 1973.

3) Levis, W.R., Whalen, J.J., and Miller, A.E.: Lymphokine production in cell mediated allergic contact dermatitis. Lancet 2: 389-390, 1973.

4) Miller, A.E., Jr., and Levis, W.R.: Studies on the contact sensitization of man with simple chemicals: I. Specific lymphocyte transformation in response to Dinitrochlorobenzene sensitization. J. Invest. Dermatol. 61: 261-269, 1973.

5) Levis, W.R., Whalen, J.J., and Miller, A.E., Jr.: Studies on the contact sensitization of man with simple chemicals: II. Lymphokine production in allergic contact dermatitis to Dinitrochlorobenzene. J. Invest. Dermatol. 62: 2-6, 1973.

6) Levis, W.R., Kraemer, K.H., Klingler, W.G., Peck, G.L. and Terry, W.D.: Topical immunotherapy of basal cell carcinomas with Dinitrochlorobenzene. Cancer Res. 33: 3036-3042, 1973.

7) Levis, W.R., Whalen, J.J., and Miller, A.E., Jr.: Blastogenesis of autologous, allogeneic, and syngeneic (Identical Twins) lymphocytes in response to lymphokines generated in Dinitrochlorobenzene sensitive human leukocyte cultures. J. Immunol. 112:1488-1493, 1974.

8) Levis, W.R., Whalen, J.J., and Sherins, R.J.: Sperm-induced lymphocyte transformation. Lancet 2: 954-955, 1974.

9) Levis, W.R., Whalen, J.J., and Powell, J.A.: Studies on the contact sensitization of man with simple chemicals III. Quantitative relationships between specific lymphocyte transformation, skin sensitivity and lymphokine activity. J. Invest. Dermatol. (In press).

10) Powell, J.A., Whalen, J.J., Levis, W.R.: Studies on the contact sensitization of man with simple chemicals IV. Timing of skin reactivity, lymphokine production, and blastogenesis following rechallenge with dinitrochlorobenzene using an automated microassay. J. Invest. Dermatol. (in press)

11) Levis, W.R., Powell, J.A., and Whalen, J.J.: Hapten vs carrier specificity in contact sensitivity. (Submitted for publication)

12) Levis, W.R., Whalen, J.J., and Powell, J.A.: Specific blastogenesis and lymphokine production in DNCB-sensitive human leukocyte cultures stimulated with soluble and particulate DNP containing antigens. (Submitted for publication)

13) Levis, W.R., Whalen, J.J., and Shevins, R.J.: Mixed sperm/leukocyte cultures as a measure of histocompatibility in man. (Submitted for publication)

Project No. Z01 CBD 3654-02 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Development and Application of Approaches and Methods of Scanning Electron Microscopy (SEM) in Studies of Soft Biological Tissues

Previous Serial Number:

Principal Investigator: Bruce Wetzel, Ph.D.

Other Investigators: Grace B. Cannon, Ph.D., Edwina Westbrook, Diana Berard, Katherine Sanford, Ph.D., Cecil Fox, Ph.D., Gary Jones, Saul Roseman, Ph.D., Paul Howard, Richard Blanquet, Ph.D., Wilmar Jansma, Ph.D., Robert Tarone.

Cooperating Units: Litton Bionetics Research Laboratories, Rockville, Md., Laboratory of Biochemistry, NCI, Department of Biology, Johns Hopkins University, Dept. of Biology, Georgetown University, and Dept. of Pathobiology, School of Hygiene and Public Health, Johns Hopkins University.

Man Years (July 1, 1974 through June 30, 1975)

Total:	2.75
Professional:	0.75
Other	2.0

Project Description:

Objectives:

To discover and develop new methods and interpretive criteria for the application of SEM to a wide variety of soft biological tissues, and thereby capitalize on the unique capabilities of this instrument in providing 1) improved survey and sampling as an adjunct to other studies, 2) an unparalleled three-dimensional, experimental view of cell form and surface phenomena, and 3) an unusual potential for cytochemical studies.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.5: Study cell surface and cell membranes.

Methods Employed:

For most studies described herein, tissue culture cells are grown as monolayers on glass coverslips, fixed by the addition of an equal volume of buffered 6% glutaraldehyde to the medium and processed appropriately for SEM. To evaluate the impact of environmental conditions on cell form and surface morphology, HBT-3 cells (a human breast tumor line) were grown on various substrates, in various media and buffered salt solutions, and in media supplemented with various concentrations of fetal calf serum (FCS).

Studies of cellular topography in relation both to interphase stages of the cell cycle and to neoplastic state were carried out on established mouse fetal lines derived from a common single cell. Some of these lines had undergone "spontaneous" neoplastic transformation as judged by the production of malignant transplantable tumors in compatible hosts; the cells had been filmed continuously by time-lapse cinephotomicrography for two days prior to fixation, and the same individual cells were relocated for SEM.

For adhesion studies, specially treated Sephadex beads or 3T3 tissue culture cells in forced suspension were allowed to settle on a monolayer of 3T3 cells for short periods of time, then fixed and rinsed in various ways.

As a more rigorous test of our methods and specimen handling procedures for the SEM, two types of delicate invertebrates (scyphopolyps of the sea nettle, Chrysaora quinquecirrha, and the hematoparasite Schistosoma mansoni) were prepared and their fine surface features surveyed.

Preliminary experiments using the backscatter mode of the SEM for cytochemistry have visualized ingested colloidal gold and iron, an osmiophilic peroxidase marker, and a variety of heavy metal stains.

Major Findings:

During the first 24 hours after seeding, the form and surface morphology of the human tumor line HBT-3 is profoundly influenced in distinctive ways by the time in culture, the nature of the underlying substrate, and the concentration of supplemental serum in the medium. Under our control conditions (on glass coverslips with 16.7% FCS in RPMI-1640 medium) the cells adhere and spread in progressively greater numbers throughout the first twelve hours and grow into small colonies of contiguous cells. Individual cells seem to flatten by spreading a thin velum of cytoplasm onto the substrate around their bases beyond the spherical contour of the cell. Grown for 24 hours on glass, many cells are thinly spread but on the adjacent surface of the polystyrene petri dish such thinly spread cells are rare. On Millipore filters of cellulose acetate in the same dish nearly all cells are characteristically spindle-shaped and remain separate despite reasonable growth. Few cells adhere to Nuclepore (Polycarbonate) filters in the same culture dish and these are mostly spherical and covered with cytoplasmic blebs. Most cells which contact Flotronic (silver) filters die within one hour.

Grown for 24 hours on glass HBT-3 cells survive poorly in RPMI-1640 and

NCTC-135 media without supplemental serum and most are dead by 12 hours in buffered salt solutions lacking serum; only 1.7% supplemental serum allows growth in these media, but a distinctive reticulate pattern supplants the small independent colonies of contiguous cells seen with 16.7% supplemental serum. The appearance of cells grown for 24 hours on glass coverslips with 16.7% serum is remarkably similar in a variety of media. We have tested RPMI-1640, NCTC-135, McCoy's Regular 5A, McCoy's 5A Spinner Modification, Eagle's Minimal Essential Medium, and even phosphate-buffered salt solutions with serum, and the cells' appearance is similar.

Cinephotomicrography and SEM of the same individual cultured mouse cells provides the best evidence to date that surface morphology is independent of interphase stages in the cell cycle. The time elapsed between the last mitosis and fixation can be accurately determined from the films, and with this one can estimate the probabilities that a given cell is in G_1, S, or G_2. Beyond 30 minutes after mitosis, surface features such as roundness, microvilli, cytoplasmic blebs, and ruffles showed no correlation with postmitotic time. Filming does not detectably perturb the cultures, and this approach avoids the severe treatment required to synchronize cell populations for study of the cell cycle.

Having established that interphase cell topography was not dependent on the cell cycle, we could compare cells of non-neoplastic and neoplastic lines despite their differences in mitotic behavior. Estimates of the incidence of microvilli on the exposed surfaces of individual cells revealed no significant difference between non-neoplastic and neoplastic lines of common single cell origin grown under identical culture conditions. The incidence of ruffles and cytoplasmic blebs, and the degree of heterogeneity of cell size and shape vary somewhat, but these features do not correlate with neoplastic state. On the other hand, non-neoplastic cells tend to spread and flatten more on the coverslip than neoplastic cells, and may show fewer lateral processes than seen in some neoplastic lines.

3T3 cells from suspension cannot be distinguished morphologically from similar forms which occur naturally in the homologous monolayers to which they adhere. Some cells from suspension have been positively identified by visualizing ingested colloidal gold in the backscatter mode. Sephadex beads adherent to the monolayer within 5 minutes generally contact only one cell through a small number of remarkably delicate processes or ruffles.

SEM studies of invertebrate surface ultrastructure in the synphopolyp and schistosome revealed very well preserved features such as cilia, microvilli, sensory end bulbs, and delicate glycogen storage tubercles. All of these features have been misrepresented and misinterpreted in previous reports due to artifactual collapse during fixation, dehydration, and drying.

All of the materials examined as potential labels for SEM in the backscatter mode have proven useful in preliminary experiments.

Significance to Cancer Research:

SEM reveals fine surface structure with unparalleled three-dimensionality, depth of field, and sampling capability. As this approach is adapted to biological problems through experience with diverse tissues, cellular form, surface details and interactions can be better interpreted in normal and malignant tissues, as well as in experimental systems which help define immune responses, differentiation, and other processes fundamental to oncogenesis and its control. The tissue culture studies directly elucidate new aspects of tumor cell biology and transformation.

Proposed Course of Project:

To further develop and refine techniques of specimen preparation, analysis, and interpretation for SEM will be the goal of continuing work with a wide variety of tissues, experimental approaches, and instrumentation. Special emphasis will be accorded a search for novel cytochemical methods, especially with labels visualized in the backscatter mode, which are uniquely possible for this tool. Further collaboration in SEM, TEM and cytochemical studies of skin is under way with investigators in this branch and elsewhere.

Keyword Descriptors:

SEM, cell surface, tissue culture, mitotic cycle, cell cycle, neoplastic state, tissue culture conditions, spontaneous transformants, cinephotomicrography, effect of serum concentration, cell adherence, tissue culture media.

Honors and Awards: None

Publications:

1) Blanquet, R.S. and Wetzel, B.: The surface ultrastructure of the scyphopolyp, Chrysaora quinquecirrha, as revealed by scanning electron microscopy. Biol. Bull. (In press)

2) Westbrook, E., Wetzel, B., Cannon, G., and Berard, D.: The impact of culture conditions on the surface morphology of cells in vitro. In Scanning Electron Microscopy/1975. (Johari, O., and Corvin, I., Eds.), IIT Research Institute, Chicago, Il. pp. 351-357.

3) Wetzel, B., Jones, G., Westbrook, E., Fox, C., Sanford, K.: Dynamic surface features of cultured cells visualized by combining cinephotomicrography and SEM. Proceedings of the 33rd Annual Meeting of the Electron microscopy Society of America, C. Arceneaux, ed. Claitor's Publishing Division, Baton Rouge, LA (in press).

4) Superior 2 X 2 slides, Harry Schaefer and Bruce Wetzel, ibid., (in press).

5) Peck, G.L., Kraemer, K.H., Wetzel, B., Klingler, W.G., and Cohen, I.K.: Cornifying Darier's disease -- a unique variant. J. Invest. Derm. (In press).

Project No. Z01 CBD 3655-04 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Treatment of Basal Cell Carcinoma with Topical Vitamin A Acid

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total:	0.02
Professional:	0.02
Others	0

Project Description:

Objectives:

To evaluate safety and effectiveness of topical Vitamin A Acid in the treatment of patients with basal cell carcinoma.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Agents:

0.5% to 3.0% Vitamin A Acid in water washable ointment base (unibase).

Material:

1) Basal Cell Carcinoma

2) Nevoid Basal Cell Carcinoma Syndrome

3) Xeroderma Pigmentosum With Cutaneous Carcinoma

Methods Employed:

1) Patients must have pathologic confirmation of the clinical diagnosis.

2) They must have normal hematologic, hepatic, and renal function.

3) Lesions on eyelids and canthi are not included.

4) Patients are cautioned to avoid sun.

5) Ointments are applied twice daily to affected areas for 8 weeks.

6) Laboratory tests are done before, during, and 6 weeks after therapy.

7) Serial photographs are taken.

Major Findings:

1) In one patient, a 4 X 2 recurrent basal cell epithelioma resolved following treatment with topically applied 1% Vitamin A Acid. The lesion had been pretreated with superficial liquid Mitrogen cryotherapy 8 weeks previously. A hypertrophic scar developed at this site which involuted after 9 months. No recurrence has developed during the 3 year observation period.

2) In a second patient, 3% Vitamin A Acid was used to successfully treat a nodular basal cell carcinoma on the dorsum of the right hand.

3) In a third patient, a solitary large keratoacanthoma resolved clinically with the combination treatment of topical 1% Vitamin A Acid and 5% 5-fluorouracil. The lesion recurred, however, within a few months.

4) The back of a patient with xeroderma pigmentosum was divided in 4 quarters and treated with a) topical 5-FU alone, b) topical Vitamin A Acid alone, c) both agents, or d) neither agent. Results indicated that the combination of agents produced the most inflammation and resulted in the most loss of pigmentation. The cancer prophylactic value of this regimen has not yet been determined as no tumors have yet developed in any of the 4 quadrants.

5) A patient with multiple actinic keratoses was treated with either a) topical 5-FU alone, b) topical Vitamin A Acid alone, or c) a combination of both agents. Results of all three treatment programs were similar in producing partial responses.

6) A patient with the Basal Cell Nevus Syndrome is being treated with the combination of topical Vitamin A Acid and topical 5-FU. Most tumors have responded well, a few have disappeared completely.

7) A patient with a solitary large superficial BCE was treated for three weeks with the combination of topical Vitamin A Acid and topical

5-FU. This lesion underwent complete clinical and histological involutic

Significance to Cancer Research:

1) This is an exploratory study of a new drug in skin cancer prompted by a desire to find a superior agent to 5-Fluorouracil in propylene glycol which is in widespread topical use.

2. We would also hope to contribute to the knowledge of the clinical pharmacology of this agent. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which would allow for long term studies.

Proposed Course of Project:

1) Continued observation of test subjects to determine recurrence rate.

2) Continued study of new patients as they become available.

Keyword Descriptors:

Basal Cell Carcinoma, Topical Vitamin A Acid, evaluate safety and effectiveness, superficial liquid Mitrogen cryotherapy, hypertrophic scar, multiple actinic keratoses, Basal Cell Nevus Syndrome, topical 5-Fluorouracil.

Honors and Awards: None

Publications:

1) Peck, G.L.: Hypertrophic scar after cryotherapy and topical tretinoin. Arch Derm 108:819-822, 1973.

Project No. Z01 CBD 3656-02 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Chemistry and Biosynthesis of Mammalian Epidermal Tonofilaments

Previous Serial Number: None

Principal Investigator: Peter M. Steinert, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total:	2.2
Professional:	1.0
Others:	1.2

Project Description:

Objectives:

1) To characterize the structure of epidermal tonofilaments isolated from bovine epidermis.

2) To investigate the mechanism by which the isolated polypeptide chain subunits of the tonofilaments aggregate into tonofilament-like filaments.

3) To characterize the polypeptide composition of the tonofilaments obtained from the epidermis of various benign and malignant diseases in human subjects.

4) To investigate the biosynthesis of the tonofilament polypeptide subunits in cell cultures of mouse epidermal cells.

5) To investigate the effect of various drugs and carcinogens on the biosynthesis of the tonofilaments in the mouse epidermal cell cultures.

Major Findings and Methods Employed:

1) Intact tonofilaments of bovine epidermis are extracted by homogenization of the tissue in sodium citrate buffer, pH 3, and purified by differential centrifugation. The structure of the tonofilaments is being

probed by both electron microscopy and x-ray difraction.

2) Extraction of bovine epidermis with an 8M-urea buffer, pH 9, dissociates the tonofilaments and releases their constituent polypeptide chain subunits.

3) Analysis of the intact tonofilaments and extracted polypeptides on polyacrylamide gels containing 6M-urea or SDS indicates that there are 7 different polypeptide chains.

4) These polypeptide chains have been separated by a combination of chromatography on DEAE-cellulose and preparative polyacrylamide gel electrophoresis. The chains can be classified into three groups on the basis of close similarities in such properties as α-helix content, molecular weight and amino acid composition.

5) The isolated polypeptide chains show a marked propensity for aggregation into filamentous structures which appear identical to the tonofilaments seen in vitro or in situ by electron microscopy. This affords an opportunity to study the mechanism of aggregation and structure of the filaments. Mixtures of the separated chains are prepared which are monitored for filament formation by high speed centrifugation, electron microscopy and x-ray diffraction.

6) Diseased (benign or malignant) epidermis from human subjects is extracted with the urea buffer to isolate the tonofilament polypeptide chains. Comparisons of the polyacrylamide-gel electrophoretic profiles of these polypeptides with those obtained from normal epidermis will provide useful information on the diseases tissue.

7) Methods have been published for the successful long-term culture of mouse epidermal cells in vitro.

(a) Polypeptide chains of the tonofilaments in these cells will be labelled with tritiated precursors in vitro, isolated with the urea buffer and used for studying the aggregation and structure of the tonofilament-like filaments.

(b) Polyribosomes and other co-factors will be isolated from these cells in order to investigate aspects of the biosynthesis of the tonofilament polypeptide chains. In particular, attempts will be made to isolate active RNA fractions by standard techniques that are capable of directing the synthesis of the polypeptide chains in vitro.

(c) The effects of such reagents as Vitamin A acid and carcinogens such as dimethylbenz-a-anthracene on the biosynthesis of the tonofilaments will be investigated using the cultured cells.

Significance to Cancer Research:

The epidermis offers a unique opportunity for the study of tumors not only because of the quantitative importance of this tissue but also because of its accessibility. One of the major problems in studying malignancies of the epidermis has been the lack of a suitable biochemical marker. The tonofilaments are the most prominent intracellular components of all epidermal cells and therefore a study of their chemistry, structure and biosynthesis in both normal and abnormal epidermis will be of profound importance in studying tumors in this tissue.

Proposed Course of Project:

Studies on the diseased tissues will be done in collaboration with Dr. Gary Peck, of this Branch. It is planned to train his technician to perform the polyacrylamide gel experiments. The investigations of the structure and assembly of the tonofilaments and the tonofilament-like filaments will be completed before detailed studies on the biosynthesis of the tonofilaments are initiated.

Keyword Descriptors:

Normal and abnormal epidermis, tonofilaments, structure and biosynthesis.

Honors and Awards: None

Publications:

1) Steinert, P.M.: The extraction and characterization of bovine epidermal α-keratin. Biochem. J. (1975) in press.

2) Steinert, P.M., and Idler, W.W.: The subunit composition of bovine epidermal α-keratin. Submitted for publication.

3) Steinert, P.M.: The heterogeneity of bovine epidermal α-keratin. J. Invest. Dermatol. (1975) in press.

Project No. Z01 CBD 3657-01 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunopathologic mechanisms involved in blistering skin diseases

Previous Serial Number: None

Principal Investigator: Stephen I. Katz, M.D., Ph.D.

Other Investigators: Kenneth C. Hertz, M.D., Hideo Yaoita, M.D.

Cooperating Units: Metabolism Branch, NCI

✓ Man Years (July 1, 1975 through June 30, 1975)

Total:
Professional:
Other:

Project Description:

Objectives:

1) To investigate the mechanisms involved in the expression of certain immunologic skin diseases, namely pemphigus, bullous pemphigoid, dermatitis herpetiformis and herpes gestationis.

2) To determine the cell surface characteristics of the cells involved in these diseases.

3) To determine whether or not genetic factors are important in the developments of these diseases.

4) To determine the ultrastructural localization of antibodies in blistering skin diseases.

Material:

Skin biopsies, either punch of Castroviejo keratome slices will be used. Also the small intestine of patients with dermatitis herpetiformis will be studied in order to determine their binding characteristics.

Methods Employed:

Direct and Indirect Immunofluorescence Cell Mediated Cytotoxicity, Immunochemical methods for identifying immunoglobulins and immune complexes. Radioimmunoassays.

Major Findings:

As this laboratory has only recently become equipped most of the studies have only recently been initiated.

Significance to Cancer Research:

A basic understanding of immunologic injury in various autoimmune disease states is important in interpreting and further current concepts in self-recognition. Pemphigus is associated with thymoma and myasthemia gravis and its study may provide a clue as to the association between pemphigus and other malignancies.

Proposed Course of Project:

Outlined above.

Keyword Descriptors:

Immunologic skin diseases, pemphigus, bullous pemphigoid, dermatitis herpetiformis and herpes gestationis, autoimmune, genetic factors.

Honors and Awards:

None

Publications:

None

Project No. Z01 CBD 3658-01 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Search for Animal Models for Genetic Skin Diseases

Previous Serial No.: None

Principal Investigator: Marvin A. Lutzner, M.D.

Other Investigators: None

Cooperating Units: Dr. Carl Hansen, Ph.D. (Division of Research Services, Veterinary Resources Branch)

Man Years: (July 1, 1974 through June 30, 1975)

Total: 2.25
Professional: 1
Other: 1.25

Project Description:

Objectives:

To seek out animal models for human genetic skin diseases. Such mutations have occurred spontaneously or been produced in various animal production laboratories; some in our own animal laboratories at NIH.

Materials:

1) The Vibrissaeless (vb) rat, an autosomal recessive mutation, produced by multiple generation X-irradiation.

✓ 2) A mutation in the mouse occurring spontaneously called (ME) which appears to have an immunological abnormality manifested by runt syndrome and early death.

✓ 3) A mouse mutant, which loses its hair in peculiar patterns and develops an inflammatory disease of the eyes.

4) A rat mutant in which the animals' eyes and snout are involved in an inflammatory disease.

5) A mutant mouse, in which the immunologic, endocrinologic and neurologic system are all involved.

Methods Employed:

Animals are bred either by heterozygote or homozygote matings. Some of the animals are kept in germ-free conditions, especially those that present immunologic defects. Tissues from these animals are studied by light and electron microscopy, by immunofluorescence, and by chemical and microbiological techniques.

Major Findings:

1) In the vibrissaeless rat it has been found that animals lose their hair at an early age and develop both spontaneous and traumatic blisters. Blisters can be produced by rubbing the skin. Histologically, separation occurs above the basal cell layer; therefore this is a suprabasalar blistering disease. Although there are several human diseases which resemble this defect, the rat does not appear to be an exace model for any known human genetic disease. Immunofluorescent studies have shown that this is not similar to pemphigus; collagen and collaginase studies have been normal; and animals have been found not to respond to systemic steroid therapy. The biochemical defect which is occurring in these rats has not been uncovered as yet.

2) The ME mouse: Since these animals die at a very early age, detailed immunologic studies have been impossible. Some of the animals have small thymuses and absent Pyer's patches. We are attempting to breed these animals and so far have not been successful in building a large enough colony to do more experimental work.

3) The MD mouse: Histologic studies are being performed attempting to delineate the defect. Microbiological studies are also being done. No major findings as yet.

4) The Masked rat: Two organisms have been isolated from the urine and eyes of these rats. They are both pasturella organisms and Koch's postulates have been satisfied in showing that these organisms are etiologically involved in producing the scaling inflammatory disease which occurs on the eyes and the snouts. The genetic basis of the susceptibility of these organisms has not yet been determined.

5) The Lethargic mouse: This is a mouse which has neurologic, endocrinological and immunologic abnormalities. No explanation has yet been found.

Significance to Biomedical Research and the Program of the Institute:

Many genetic diseases of the skin occur in which there is no knowledge of cause or treatment. These diseases are rare and experimentation is not easily performed on humans; therefore, it is of interest to try to develop or discover animal models for these diseases in the hope that when such animals have been found it will be possible to find the cause and treatment for these genetic abnormalities which would be hopefully translatable to human disease.

Proposed Course of Project:

Continuation of research as indicated.

Keyword Descriptors:

Vibrissaeless, genetic blistering disease, animal model for human genetic disease.

Honors and Awards:

None

Publications:

1) Lutzner, M.A., and Hansen, C.T.: Skin blisters and hair loss in a rat mutant called Vibrissaeless. J. Invest. Dermatol. (In press)

Project No. Z01 CBD 3659-01 D
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Treatment of Disorders of Keratinization with Alpha-Hydroxy Acids

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1974 through June 30, 1975)

Total:	0.02
Professional:	0.02
Other:	--

Project Description:

Objectives:

To evaluate safety and effectiveness of topical alpha hydroxy acids in the treatment of ichthyosis, psoriasis, Darier's Disease, and other disorders of keratinization.

Agents:

5% Lactic Acid, pyruvic acid, methyl pyruvate, ethyl pyruvate, tartaric acid or glycolic acid in water washable ointment base.

Material:

1) Ichthyosis
    a) lanallar ichthyosis
    b) X-linked ichthyosis
    c) ichthyosis vulgaris
2) Darier's disease
3) Psoriasis
4) Keratoderma palmaris et plantaris

Methods Employed:

1) Medication will be applied twice daily for up to 6 weeks or until

clear. Occlusive dressings may be used at bedtime.

2) Serial photographs will be obtained.

Major Findings:

1) 80F Psoriasis-scalp - no effect - 3 weeks - methyl pyruvate.
2) 45F Darier's Disease-feet - moderate reduction of hyperkeratosis-glycolic acid.
3) 45F Darier's Disease-thighs - no effect in minimally hyperkeratotic lesions.
4) 45M Darier's Disease-thighs and chest - moderate reduction of hyperatosis with methyl and ethyl pyruvate.
5) 18M Darier's Disease-chest - no effect on minimally hyperkeratotic lesions with lactic acid.
6) 4M X-linked ichthyosis - complete clearing in 3-4 days. Total body-with glycolic acid - No recurrence in 3-4 weeks.
7) 4F lamellar ichthyosis-neck and shoulders - no effect with ethyl pyruvate without occlusion for 3 weeks.
8) 25F lamellar ichthyosis-total body - moderate reduction of hyperkeratosis using glycolic acid without occlusion. Complete clearing of hyperkeratosis using lactic acid with occlusion - beginning after 1 day of therapy - recurrence seen 4 days after discontinuation.
9) 25M Keratoderma palmaris et plantaris. Recently begun on pyruvic acid and occlusive dressing. No effects yet.

Significance to Cancer Research:

The alpha-hydroxy acids which are proving to be keratolytic agents may enhance the antineoplastic effect of chemotherapeutic agents when applied topically in combination.

Proposed Course of Project:

Continued testing of these agents in hyperkeratotic disorders.

Keyword Descriptors:

✓ Topical alpha-hydroxy acids, psoriasis, Darier's Disease, lactic acid, pyruvic acid, pyruvate, ethyl pyruvate, glycolic acid.

Honors and Awards: None

Publications: None

ANNUAL REPORT OF THE METABOLISM BRANCH
SUMMARY OF SIGNIFICANT ACTIVITIES
NATIONAL CANCER INSTITUTE

July 1, 1974 through June 30, 1975

The clinical research program of the Metabolism Branch is directed toward two major goals. The first is to define host factors that result in a high incidence of neoplasia. In this area, a broad range of immunological investigations are carried out in patients with malignancy, in patients with immune deficiency diseases and a high incidence of neoplasms as well as in experimental animal systems to determine the nature of the immune processes that are of importance in the surveillance against neoplastic cells and to define the nature of defects in the immunological system that result in an increased incidence of neoplasia. Major efforts in this area are also directed toward developing techniques for the detection of circulating tumor associated antigens that may be of value in the diagnosis of malignancy. The second major goal of the Branch is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Both patients with neoplastic disease as well as those with non-neoplastic disorders that facilitate the development of techniques for the study of cell membranes, homeostatic mechanisms and metabolic derrangements of these control mechanisms are being investigated.

Detection of Circulating Tumor Associated Antigens

Sensitive quantitative radioimmunoassays for oncofetal proteins such as alpha-fetoprotein (AFP) and the beta chain of human chorionic gonadotropin (hCG-β) that were established in previous years have been evaluated as tests to assist in the diagnosis of certain forms of malignancy and in the monitoring of the effectiveness of therapy of different forms of cancer. An elevated AFP (> 40 ng/ml) was demonstrated in a significant number of the over 1000 patients with cancer studied including over 70% of the patients with hepatocellular carcinoma or with teratocarcinoma or embryonal cell carcinoma of the testis. In addition, a lesser percentage of patients with entodermal tumors of other organs such as the pancreas, stomach, colon or lung had elevated alpha-fetoprotein levels. In contrast only one of 600 controls or patients with chronic nonhepatic diseases had a serum level over 40 ng/ml. In addition, only one of 300 patients with tumors of nonentodermal origin such as tumors of the breast, kidney and prostate had an elevated alpha-fetoprotein level.

The radioimmunoassay for alpha-fetoprotein used in conjunction with the assay for hCG-β was of special value in monitoring the effectiveness of therapy of certain forms of malignancy (e.g., embryonal cell carcinoma or teratocarcinoma of the testis) since the products of a few tumor cells were detectable with this assay when they were not detectable when assessed with simple agar diffusion tests. Ninety percent of the over 100 patients with embryonal cell carcinoma or teratocarcinoma of the testis studied had an elevated level of at least one of the marker proteins. Following cancer chemotherapy, there was a reduction of both markers initially. In over 50% of the cases, however, although both markers declined only one of the markers remained elevated and the remaining marker fell to normal levels. With recurrence of the tumor one of the markers remained at the normal level, whereas the other returned to a very high level. This discordant behavior of these tumor markers suggests that their cells of origin are probably different and underscores the need to follow both markers in evaluating the adequacy of therapy. The importance of following both markers in evaluation of the adequacy of therapy is also supported by the observation that in over 30% of the treated patients in the present group there was an elevation of one of the markers at a time following therapy when there was no clinical evidence of residual disease. In each of these cases with an elevation of tumor marker there was a recurrence of the tumor within the subsequent year. Thus, it is our view that these markers should be used in all protocol studies in which patients with testicular tumors are treated and that the presence of a positive marker should be taken into consideration in determining the future course of the patient's therapy.

Studies of alpha-fetoprotein in patients with benign liver disease were continued. In these studies it was shown that approximately 20% of patients with benign liver disorders have elevated alpha-fetoprotein levels. Concentrations were more frequently elevated in disorders characterized by a prominent parenchimal necrosis, inflammation and extensive liver regeneration. In following patients with active hepatitis there was no positive correlation of an elevated alpha-fetoprotein level in the serum with determinations of the other hepatic function tests that measure hepatic necrosis, blood flow or protein synthesis. However, in patients with infectious hepatitis the alpha-fetoprotein levels become elevated as the serum SGOT was declining. These observations taken in conjunction with known features of alpha-fetoprotein synthesis in partially hepatectomized animals support our view that the alpha-fetoprotein synthesis measures liver regeneration, a function not assessed by other clinical tests.

Immunophysiology and Clinical Immunology

A significant effort of the Metabolism Branch over the past years has been directed toward defining the nature of the events of cellular differentiation, cellular interactions and cellular biosynthesis involved in the specific circulatory and cellular immune responses to antigens. The first group of these studies are directed at defining the factors controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo a sequential maturational process from stem cells originating in the marrow. The first step that does not require antigen but requires a central lymphoid organ involves the maturation of marrow stem cells into B lymphocytes,whereas the second phase which is either antigen or mitogen driven involves terminal differentiation of B lymphocytes into cells that synthesize immunoglobulins and antibodies. The majority of patients with immunodeficiency diseases of man appear to have a defect in the terminal differentiation of B lymphocytes into immunoglobulin synthesizing and secreting cells. We have, therefore, developed a technique for the study of the terminal differentiation of such B lymphocytes to define the nature of this critical process and define defects in this process in patients with immunodeficiency disorders. In these studies we have laid special emphasis on the role of regulatory T cells in the control of this maturational event. In the technique utilized, peripheral blood lymphocytes from patients and controls are washed free of serum immunoglobulin and incubated in culture media for a 7-day period in the presence of pokeweed mitogen. The IgM, IgA, and IgG molecules synthesized and secreted by these cells into the media are then quantitated using sensitive double antibody radioimmunoassays for each of these immunoglobulin classes.

In normal individuals, the terminal B cell maturational process can be triggered by the pokeweed mitogen. The peripheral blood lymphocytes of normal individuals synthesize 4900 ng of IgM, 1260 ng of IgA, and 1625 ng of IgG per 2 million lymphocytes cultured for 7 days in the presence of pokeweed mitogen. Pokeweed mitogen was the only chemical agent to cause a marked and consistent stimulation of maturation and synthesis by B cells. However, a number of physiological factors were shown to inhibit this maturational process. Dibutyryl cyclic AMP or factors which increase intracellular cyclic AMP such as isoproteranol and prostaglandin E_1 prevented B cell maturation and immunoglobulin synthesis. HCG-β and alpha-fetoprotein also inhibited this process. In addition, we have obtained evidence that tissue specific but species nonspecific inhibitors of B cell maturation exist (i.e., chalones). In these studies 30-50,000 molecular weight molecules extracted from plasmacytomas of mice with associated hypogammaglobulinemia markedly inhibited the terminal differentiation and immunoglobulin biosynthesis of pokeweed mitogen stimulated human B cells. These materials did not inhibit fibroblast protein synthesis or proliferation. Other materials which inhibit B cell maturation include the mitogens, phytohemagglutinin, staphylococcal filtrate or concanavalin A. That is, when these agents were added to the normal cultures of the lymphocytes in optimal mitogenic doses in the presence of pokeweed mitogen, immunoglobulin synthesis was depressed to 0-16% of normal. Preliminary studies suggest that two mechanisms are of significance in this inhibitory process. The first mechanism is a direct inhibitory action of these soluble mitogens on the B cells. B cells and T cells have equal number of receptors for phytohemagglutinin and concanavalin A. Using the present immunoglobulin biosynthesis procedure we have shown that concanavalin A and phytohemagglutinin inhibit the immunoglobulin synthesis of purified B cells. These studies suggest that these agents in soluble form may act to interfere with immunoglobulin production by B cells by interacting directly with the receptors for these mitogens on the B cells, leading to an inhibitory signal to these cells. We have, in addition, developed preliminary evidence supporting the view that a subset of the population of T cells can be activated by concanavalin A to non-specifically suppress antibody production by B cells.

The technique for the quantitation of in vitro immunoglobulin biosynthesis and secretion was applied to the study of peripheral blood lymphocytes of 22 patients with common variable hypogammaglobulinemia. Four of these patients were able to make small quantities of IgM in vitro when cultured in the presence of fetal calf serum alone. When cultured in their own serum alone or in their own serum plus added fetal calf serum, synthesis of immunoglobulin in this subset of patients was markedly inhibited. In addition, immunoglobulin synthesis by normal cells cultured in the serum of these patients were markedly inhibited. These studies suggest that in this small subset of patients with common variable hypogammaglobulinemia there is a circulating inhibitor of B cell maturation and immunoglobulin synthesis. Lymphocytes of the remaining 18 patients with this disorder studied were not able to make significant quantities of IgA, IgG or IgM in vitro in the presence or absence of pokeweed mitogen.

To determine whether the failure of lymphocyte immunoglobulin synthesis by this second group of patients with hypogammaglobulinemia was due solely to an intrinsic defect of the maturation of their B lymphocytes or whether it could be due to the presence of suppressor cells, the lymphocytes of patients with common variable hypogammaglobulinemia were co-cultured with lymphocytes of normal individuals. When the lymphocytes of the 9 out of 13 patients with common variable immunodeficiency studied by this technique were co-cultured with normal lymphocytes in the presence of pokeweed mitogen, the synthesis and secretion of immunoglobulin by the normal lymphocyte was depressed to 0-16% of normal. Incubation with purified T cells from the hypogammaglobulinemic patients resulted in a comparable suppression of the immunoglobulin synthesis by normal lymphocytes. A series of control studies were designed to determine if the cellular suppression process was a specific property of cells from patients with common variable hypogammaglobulinemia. In these studies it was found that co-culture of lymphocytes from unrelated normal individuals did not result in significant inhibition of immunoglobulin synthesis. Thus, the IgM, IgG and IgA synthesized in co-culture by lymphocytes of 20 pairs of of unrelated normal donors gave mean values between 93-106% of the expected sum of the immunoglobulins synthesized by the two individuals cultured separately. Similarly, incubation of normal lymphocytes with lymphocytes from patients with isolated IgA deficiency or the Sezary syndrome, a T cell leukemia did not inhibit immunoglobulin synthesis. Finally, there was no inhibition of immunoglobulin synthesis by the lymphocytes of 7 normal individuals when they were co-cultured with isolated T cells from unrelated normals. These studies indicate that the ability of cells of one individual to inhibit immunoglobulin biosynthesis by the cells of another is not a non-specific phenomenon but is a specific feature of the patients with common variable hypogammaglobulinemia. We investigated the capacity of purified peripheral blood B lymphocytes from a patient with common variable hypogammaglobulinemia to synthesize immunoglobulin. The unseparated lymphocytes and the purified T cells from this patient studied showed no immunoglobulin synthesis in vitro and depressed the immunoglobulin synthesis of normal lymphocytes by greater than 90%. In contrast, the purified B lymphocytes from this patient did not suppress the immunoglobulin synthesis by normal lymphocytes. In addition, these B cells synthesized normal quantities of IgM when cultured with pokeweed mitogen in vitro. These studies suggest that in a significant subset of patients with common variable hypogammaglobulinemia the defect is caused or perpetulated by an abnormality of regulatory suppressor T cells which act to inhibit B cell maturation and antibody synthesis. That is, this subset of patients did not appear to have an intrinsic defect in the maturation of their B lymphocytes, but rather had an abnormal number or abnormally activated set of suppressor T cells.

Patients with thymomas frequently have hypogammaglobulinemia and an increased incidence of infections. We have previously found that 10% of the 100 patients studied with thymoma have this associated hypogammaglobulinemia. When the immunoglobulin biosynthesis technique was applied to the study of lymphocytes from two patients with thymoma and hypogammaglobulinemia the pattern similar to that observed with common variable hypogammaglobulinemia was obtained. That is, the two patients with hypogammaglobulinemia and thymoma studied synthesized less than 100 ng of each class of immunoglobulin. When lymphocytes of these patients were co-cultured with normal lymphocytes and pokeweed mitogen the synthesis of immunoglobulin by the lymphocytes was depressed by a factor of 66 to 97%. Incubation of normal lymphocytes with purified T cells from the patients with thymoma and hypogammaglobulinemia resulted in a suppression of immunoglobulin synthesis by a factor of 73 to 100%; thus, suppressor T cells may play a role in the pathogenesis of the hypogammaglobulinemia observed in the patients with thymoma and hypogammaglobulinemia studied.

Patients with multiple myeloma have been shown to have a significant reduction in the serum levels of polyclonal immunoglobulins and in the percentage of circulating lymphocytes that have normal surface immunoglobulins. Such patients have been shown to have a reduced capacity to synthesize antibody in response to antigenic challenge and to have an increased incidence of infections with highly pathogenic encapsulated bacteria. We have applied the in vitro immunoglobulin biosynthesis measurement to the study of peripheral blood lymphocytes from 22 patients with myeloma. The lymphocytes of the patients studied showed profound depression of polyclonal (nonparaprotein class) immunoglobulin production. Thus, the geometric mean polyclonal immunoglobulin synthetic rates were 454 ng for IgM, 321 ng for IgA, and 216 ng for IgG/ 2 million lymphocytes in culture for 7 days in the presence of pokeweed mitogen. These mean values are approximately 10% of the mean values of normal individuals. Peripheral blood lymphocytes from 3 of the 6 patients with myeloma tested suppressed the polyclonal immunoglobulin synthesis of normal lymphocytes when co-cultured with them. Suppression generally range from 75 to 100%. However, in contrast to the observations in patients with common variable hypogammaglobulinemia and thymoma and hypogammaglobulinemia this suppression was not observed when purified T lymphocytes of patients with myeloma were co-cultured with lymphocytes of normal individuals. However, in the one patient studied removal of phagocytic mononuclear cells aggregated this suppressor activity. These observations suggest that one mechanism for the humoral immune deficiency observed in patients with myeloma is a block of polyclonal B cell terminal maturation by circulating suppressor cells.

We have extended our studies of the role of immunoregulatory T cells in the control of immunoglobulin synthesis by studying an experimental model system of agammaglobulinemia in chickens. Avian species have a central lymphoid organ for the humoral immune system, the bursa of Fabricius. Removal of the bursa in the immediate post-hatching period with the addition of sublethal irradiation results in the development of agammaglobulinemia when birds mature. This immune deficient state is characterized by total deletion of the humoral immune system with absence of immunoglobulins in serum and secretions, absence of plasma cells in germinal centers and lymphoid tissue and absence of B lymphocytes in the lymphoid organs in circulation. Functions of the thymic-dependent immune system remain intact in these animals. During experiments to reconstitute humoral immune responsiveness in such agammaglobulinemic birds by transplantation of bone marrow from normal syngenic donors, it was observed that immunoglobulin production could not be restored by the transplant of agammaglobulinemia birds even though cytogenetic analysis revealed successful engraftment by the cells from the normal donor. The converse experiment resulted in even more curious results. When normal birds were grafted with thymus or bone marrow cells obtained from agammaglobulinemic donors, the recipient normal birds developed agammaglobulinemia. This "infectious agammaglobulinemia" differs from that induced by bursectomy in the newly hatched period in several critical respects. Although these birds lack detectable immunoglobulin and antibody responsiveness, they continue to have "B" lymphocytes in their spleens and peripheral blood as determined by the presence of surface bound immunoglobulin detected by immunofluorescence. In addition, the bursa of Fabricius remains present in these birds and their lymphoid tissues contain some lymphoid follicles and occasionally germinal centers. Thus, the immune defect in these birds closely resembles that seen in the majority of our patients with common variable hypogammaglobulinemia. Thus, in the infectious agammaglobulinemia of chickens excessive activity of a population of suppressor T lymphocytes can be implicated in the perpetuation of the humoral immunodeficiency.

The Metabolism Branch continued their studies of cell-mediated immunity using a variety of basic model systems. Several of these systems were extended to study the state of immune competence of patients with neoplastic disease and/or immune deficiency disease. Four fundamentally different cellular immune functions have been assessed in vitro by different techniques: 1. proliferative response of lymphocytes following stimulation with mitogens, specific antigens, and allogeneic cells; 2. the production of biologically active soluble mediators (lymphokines) secreted by activated lymphocytes; 3. the cytotoxic killer cell activity of both immune and non-immune lymphoid cells and macrophages; and, 4. the in vitro responsiveness of mononuclear leukocytes (monocytes and macrophages) to various lymphokines and other biological mediators such as activated serum complement components.

One leukocyte function receiving intensive investigation is the cytotoxic effector capacity or killer activity by which lymphoid cells destroy various tumor cells and other tissue targets. Studies of the identity, characteristics and mechanisms of action of cells involved in these reactions have been a major effort of this Branch during the past year. To investigate the basic mechanisms of cytotoxic reaction, two model systems (antibody-dependent cellular cytotoxicity or ADCC and mitogen-dependent cellular cytotoxicity or MDCC) have been employed. ADCC is a cytotoxic reaction in which target cells coated with specific antibody are killed by non-immune effector lymphoid cells. The identity of the effector cell mediating ADCC has been the subject of considerable controversy with little agreement between the various laboratories involved in these studies. We have found that many cell types possess the capacity to kill various antibody coated target cells. This activity depended upon the nature of the target cell under attack and the type of antibody utilized in the system. When human HLA-7 bearing lymphocytes were utilized as targets with a human anti-HLA-7 antibody, the effector cell leading to target cell destruction was a B-lymphocyte in man. Neither T-cells, polymorphonuclear leukocytes, nor monocytes were capable of mediating cytotoxicity in this particular system. By contrast, if human group B red blood cells coated with human anti-B antibody were utilized, only monocytes and macrophages were capable of effecting these cytotoxic reactions, whereas T cells, B cells and polymorphonuclear leukocytes were not. Finally, in yet another system, if chicken red blood cells were used as targets with a rabbit hyperimmune anti-chicken red cell antibody, B cells, macrophages and monocytes, polymorphonuclear leukocytes and even non-lymphoid cell lines and tumor cells such as rat embryo fibroblasts or the P815 mouse mastocytoma cell line were capable of initiating the killer reaction. Thus, antibody dependent cell-mediated cytotoxicity can be a reaction mediated by a small well-defined group of effector cells or by a large number of different forms of effector cells including non-lymphoid cells. It is particularly noteworthy that each of the effector cells active in antibody dependent cellular cytotoxicity have a receptor for the activated Fc portion of IgG. A number of tumor cells have also been described which contain such Fc receptors and these studies have demonstrated that some of these tumor systems may themselves become cytotoxic effectors in this _in vitro_ system.

Studies of patients with neoplastic and immune deficiency disease employing assays for antibody dependent cellular cytotoxicity have revealed a number of interesting characteristics. As might be expected, patients with aplastic anemia had defective killing of human red blood cell targets in the ADCC system with human group B red blood cells as the targets, since the killing in this system is dependent on macrophage function. However, these patients have normal cytotoxic activity when assayed in ADCC systems requiring B lymphocytes as the effectors. Patients with agammaglobulinemia who lack circulating B cells have defects in that in the form of ADCC requiring B lymphocytes as a killer (i.e., that with HLA-7 bearing lymphocytes as targets), but have normal antibody dependent cellular cytotoxicity reactions in systems that utilize monocytes, polymorphonuclear leukocytes or other effector cell populations. Patients with intestinal lymphangiectasia, a protein-losing gastroenteropathy associated with the generalized lymphatic abnormalities and lymphocytopenia, were found to have deficient T lymphocyte proliferative responses in their peripheral blood. The ADCC responsiveness of peripheral blood lymphocytes was, however, greater than that of normal controls suggesting that the cell population lost by patients with intestinal lymphangiectasia does not include those cells participating in this form of cytotoxicity. Conversely, chylous effusion lymphocytes obtained from patients with this disease were found to have a normal proliferative response and a defective cytotoxic reactivity in the ADCC system for B lymphocyte killing.

Mitogen activated cellular cytotoxicity is another model system for cell-mediated target cell killing which utilizes a mixture of effector cells, target cells and various plant lectins to develop cytotoxicity. Lectins such as phytohemagglutinin, concanavalin A and pokeweed mitogen are effective in inducing the killer cell reaction to a variety of target cells. Here again we found that these assays are very dependent upon the type of target cell studied and specific lectin employed. It was found that the lectin provides a specific link between the effector cell and target cell so that the cytotoxic reaction can proceed. The lectin provides a certain degree of specificity in these reactions for both the effector and target cell must have a receptor for the lectin in order for the linking to proceed normally. Thus, concanavalin A will induce mammalian effector cells to kill burro erythrocytes which have a receptor for concanavalin A but will not induce the same effector population to kill human erythrocytes which lack a receptor for concanavalin A. Conversely, pokeweed mitogen induced killing of human erythrocytes but not of burro erythrocyte target cells. As in the system of antibody dependent cellular cytotoxocity there is specificity as to the killer cells which are effective for different classes of target cells. Metabolically active target cells such as the P815Y mouse mastocytoma tumor cell were killed only in the presence of a mixture of T lymphocytes and mitogen. Thus, cells from athymic nude mouse were unable to kill this target cell in the presence of phytohemagglutinin. However, if chicken erythrocytes were employed as the target cell, athymic nude mouse spleen cells were very effective in inducing the killer reaction of these targets in the presence of PHA. In fact erythrocyte target cells were killed by T cells, B cells, marcophages and such non-lymphoid cells as cultured mouse L cells, rat embryo fibroblasts and the P815 mastocytoma tumor cell line. Thus, similar to the heterogeneity of effector function observed in antibody dependent cellular cytotoxicity mitogen dependent cellular cytotoxicity also may reveal the effector potential of a wide variety of both lymphoid and both non-lymphoid cells.

Another major area of investigation by the Metabolism Branch has involved various mechanisms of immunoregulation. One of the most striking examples of physiologic immunoregulation occurs during pregnancy as demonstrated by the absence of immunologic attack by maternal cells on the histoincompatible placenta and fetus. Among several cooperating factors contributing to this lack of immune rejection, we have found that human chorionic gonadotropin (HCG), a glycoprotein hormone produced in vast quantities by the syncitiotrophoblast of the placenta, is a potent immunosuppressive compound. HCG in concentrations found at the junction of the placental and uterine vessels inhibited proliferative and cytotoxic effector cell activity of human peripheral blood lymphocytes. The most profound inhibitory activity on proliferative responses was noted in specific antigen induced lymphocyte responses and in mixed lymphocyte reactivity, while non-specific mitogen induced reactivity was inhibited by higher HCG concentrations. In addition, the cytotoxicity effector function of lymphoid and non-lymphoid cells was also blocked by HCG in ADCC and MDCC assays. The immunosuppressive effect of HCG was completely reversible so that cells washed free of the hormone regained normal functional capacity. Thus, HCG possesses many of the properties of an ideal immunosuppressive agent for pregnancy. It works most efficiently in inhibiting specific antigen induced immune responses; it is an efficient immunosuppressive only in concentrations found at the site of maximum desired effect (the placenta-uterine interface); it's immunosuppressive effect are completely reversible so that generalized maternal immunocompetence is not impaired; and it suppresses both the recognitive-proliferative phase and also the cytotoxic effector phase of cellular immunity.

Immunodeficiency and Malignancy

Studies of patients with immunodeficiency disease and a high incidence of malignancy including patients with the Wiskott-Aldrich syndrome, hypogammaglobulinemia, ataxia telangiectasia and intestinal lymphangiectasia are continuing with the ultimate goal of correction of the immunologic defect in these patients and gaining insights into the role of immune processes in prevention and control of malignant disease.

The Wiskott Aldrich syndrome is an x-linked immunodeficiency disorder with a very high incidence of infections with all classes of microorganisms and, in addition, the highest rate of malignant disease of any of the immunodeficiency diseases. Intensive study of these patients has demonstrated pronounced defects in both humoral and cellular immunity characterized by clinical anergy and defective antibody production. In spite of these pronounced clinical immune defects, our studies have demonstrated that the effector arms of their immune system as assayed in vitro are substantially normal. These observations have led us to postulate that the immunodeficiency in this disorder is the result of a defect in the afferent limb of immunity, as perhaps a defect in antigen recognition or processing or as a defect in the control of immune responsiveness. One of the most striking features of this syndrome is the very high incidence of lymphoreticular malignancy found in these patients. Six of the 13 patients studied at the NIH since 1966 have developed malignancy. In a very provocative study in collaboration with Drs. K. K. Takomoto and A.S. Rabson, a papovavirus has been isolated from a reticulum cell sarcoma involving the brain of one of our patients. Serum from this patient was studied for antibody to the virus and about 14 months prior to the clinical appearance of the tumor the patient developed a strong antibody response to this virus. Following the isolation of this papovavirus from the child's brain tumor, the virus was also isolated from his urine on each of three attempts. The isolation of a virus belonging to the papova group, which are well-known for their oncogenic properties from a human tumor raises intriguing questions in regard to the possible role of these viruses in human cancer. Although this single observation certainly does not prove an etiologic relationship between this virus and cancer, it does provide a strong stimulus to further investigation of its association with malignancy, particularly that occurring in immunodeficient individuals. In this connection, a similar papovavirus has been isolated from the urine of all four additional boys with the Wiskott Aldrich syndrome studied. One of these boys also had developed a lymphoma. Urines of 14 patients with other forms of immunodeficiency including ataxia telangiectasia, agammaglobulinemia and intestinal lymphangiectasia were also studied and in no case was a similar virus recovered.

A broad range of studies on the pathogenesis of gluten-sensitive enteropathy were continued. Gluten-sensitive enteropathy (GSE) is a disease of the gastrointestinal tract associated with malabsorption, villous flattening and increased incidence of neoplasia. It is due to the toxic effect of wheat protein (gluten) on the gastrointestinal mucosa. In previous studies we observed that approximately 80% of patients with GSE carry the HL-A8 histocompatibility antigen. Moreover a similar association between HL-A8 and gastrointestinal disease occurs in dermatitis herpetiformis, a skin disease associated with a gastrointestinal lesion similar to that seen in GSE. The cause of this association was initially thought to be due to a genetic linkage between the HL-A8 gene (or an alternate histocompatability gene) and the gene responsible for GSE, the GSE gene. In current studies this concept was explored by analysis of HL-A types in the families of some 35 patients with GSE. It was observed that although many siblings and parents of patients with GSE carried the same genotype and/or haplotype as the patient, GSE occurred as a very low frequency in relatives. In one family GSE occurred in 2 siblings, one bearing HL-A8 and one not bearing HL-A8; in another family GSE occurred in a grandmother and 2 of 4 grandchildren, yet neither of the grandchildren bore the HL-A antigens of the grandmother. These family studies indicate that the HL-A8 gene and the GSE gene are not on the same chromosome and the high frequency of HL-A8 in patients with GSE is not due to genetic linkage. On the contrary, these studies indicate that the HL-A8 gene itself has a direct or indirect effect on the pathologic processes leading to gluten sensitivity.

Additional insight into this problem was obtained from the studies utilizing an in vitro model of GSE previously developed in this laboratory. This model involves the organ culture of jejunal biopsy tissue and measurement of brush border alkaline phosphatase activity during culture as an indication of epithelial cell maturation. As shown previously alkaline phosphatase activity in tissue from a control patient increases during culture whether or not gluten peptides are present in the culture medium. In contrast, alkaline phosphatase in tissue obtained from patients with GSE increases during culture in the absence of gluten peptides, but does not increase during culture in the presence of gluten peptides. Thus, normal tissue matures in culture whereas GSE tissue does not mature in culture when gluten peptides are present.

Using this in vitro model of GSE it was found that tissue from a series of patients with GSE who are HL-A8 positive are inhibited during culture by α-gliadin, a purified wheat protein, or by a cyanogen bromide fragment of α-gliadin. In contrast, tissue from another series of GSE patients who are HL-A8 negative are not inhibited during culture by α-gliadin but were inhibited by the cyanogen bromide breakdown product. These studies indicate that HL-A positive and HL-A8 negative GSE patients are distinguishable in culture at a molecular level. They dovetail with the family studies in that they again indicate that the HL-A8 is not merely a marker gene genetically linked to the GSE gene, but rather that the HL-A8 gene contributes to the disease in a more active way. Since histocompatibility genes control proteins on cell surfaces, it is likely that the HL-A8 gene product contributes to the cell surface receptor allowing for the binding of wheat proteins to cells in the GI mucosa. HL-A8 negative patients have a somewhat different receptor thereby accounting for the different behavior of their tissue in organ culture.

Also using the in vitro model of GSE alluded to above we have initiated studies of the identity of the molecular configuration necessary for toxicity in GSE. In such studies we have identified a molecular fragment having a molecular weight of approximately 20,000 Daltons and a partially characterized amino acid sequence which shows toxicity for the jejunal tissue obtained for GSE patients. This material is non-toxic to control patients.

The in vitro model was also used to further clarify the processes responsible for cellular damage in GSE. In previous studies we have gathered evidence supporting the concept that the pathologic process involves an endogenous mechanism of toxicity which acts through a humoral mediator. In current studies we have shown that in vitro gluten peptide toxicity (as indicated by inhibition of alkaline phosphatase increases during culture) is blocked by the presence of steroids in the culture medium. This finding provides additional evidence that gluten peptide toxicity is not a direct toxicity but rather a toxicity that depends upon the mediation of an endogenous mechanism.

Previous studies have shown that patients with GSE respond to the ingestion of dietary gluten with the local production of anti-gluten antibody. These studies, along with the finding of an association between the HL-A8 antigen and GSE, encourages the possibility that the fundamental defect in GSE is the presence of an abnormal immune response gene. If this were so, however, one would expect patients with GSE to exhibit abnormal *in vitro* reactivity to gluten proteins. In current studies we have shown that a variety of gluten protein preparations cultured with lymphocytes obtained from GSE failed to induce proliferative responses. Moreover, parallel attempts to induce lymphokine production (migration inhibition factor) by lymphocytes from GSE patients *in vitro* were also unsuccessful. These experiments are consistent with the concept that gluten proteins are not recognized as immunogens by lymphoid cells unless presented to such cells in a special configuration. The HL-A8 gene product, by participating in the formation of a binding site for gluten in the GI tract, may facilitate the formation of such an immunogenic configuration. In this sense GSE is not due to an abnormal immune response gene but to enhance immunogenecity secondary to a binding phenomena.

During this past year a unique immunologic defect of the gastrointestinal tract was identified and extensively characterized. This defect was found in a patient with chronic intestinal moniliasis associated with minimal malabsorption. This patient proved to have normal serum IgA levels, increased numbers of circulating IgA-bearing cells and a normal capacity to produce IgA by peripheral lymphoid cells. Despite these findings the patient had greatly reduced secretory IgA (salivary and jejunal fluid IgA) and a greatly reduced capacity to synthesize IgA and jejunal tissue *in vitro*. In contrast to patients with IgA deficiency in both the circulation and secretions, this patient had no free secretory component in either saliva or jejunal secretion. These findings indicate that the patient had a normal capacity to elaborate IgA cells but such cells failed to home to (or to differentiate in) the gastrointestinal mucosa; since the patient lacked the capacity to synthesize secretory piece, this molecule may play an important role in the differentiative process of IgA cells.

Proline Transport, Biosynthesis and Metabolism

We previously showed that there are two forms of P5C reductase (p5CR) activity in mammalian cells. $\mathrm{P5CR_s}$ is sensitive to inhibition by proline (50% inhibition at [proline] = 2 x 10^{-4}M). $\mathrm{P5CR_i}$ on the other hand, is insensitive to inhibition by proline (less than 20% inhibition at [proline] = 10^{-3}).

We have shown that: 1. Adult rat liver has predominently $\mathrm{P5CR_i}$ whereas fetal liver has 2-3 times the specific activity of P5CR and the enzyme is predominantly $\mathrm{P5CR_s}$. The conversion from $\mathrm{P5CR_s}$ to $\mathrm{P5CR_i}$ with development so that the ratio is about 1:1 at 3-4 weeks of age. 2. Rapidly proliferating cells in tissue culture have only $\mathrm{P5CR_s}$. This applies to all epitheloid and fibroblast cells tested. 3. In lymphocytes undergoing phytohemagglutinin-induced blastogenesis, total P5CR activity increased 15-fold and the activity converts from $\mathrm{P5CR_i}$ to $\mathrm{P5CR_s}$. These observations suggest that accompanying cell proliferation, the activity of P5CR increases but the increased activity is sensitive to feedback inhibition by proline. We are attempting to separate the putative P5CR isozymes on acrylamide gel electrophoresis. Three peaks have been identified. We are presently quantitating the activities in these peaks and characterizing the activities with respect to proline sensitivity.

We have characterized the genetic defect in type II hyperprolinemia as an absence of P-5-C dehydrogenase (P5CD). Carriers for the defect may be identified by studying P5CD activity in peripheral leukocytes. The transmission of the defect is autosomal recessive. Since patients with the disorder excrete 3-OH-P-5-C as well as P-5-C,we considered the possibility that these respective intermediates in the degradation of hydroxyproline and proline may by dehydrogenated by a single enzyme. We enzymatically synthesized and purified radioactively labeled 3-OH-P5C and assayed for conversion of 3-OH-P5C to γ-OH-glutamate in normal and type II hyperprolinemia fibroblasts. The activity in extracts from normal fibroblasts was linear with increasing time and extract protein and was inhibited by P-5-C. In contrast 3-OH-P5C dehydrogenase activity was undetectable in type II hyperprolinemic fibroblasts. This absence of 3-OH-P5C dehydrogenase activity was not due to the presence of an inhibitor, since the activity in extracts of normal fibroblasts was not significantly inhibited by the extract from type II hyperprolinemia fibroblasts. These studies suggest that 3-OH-P5C and P5C are oxidized by a single enzyme which is absent in type II hyperprolinemia.

We are studying the enzyme, proline oxidase, which converts proline to P-5-C. The enzyme is undetectable in most cultured cell lines but is found in certain but not all transformed cell lines. The highest levels, however, were found in normal rabbit kidney cells (LLC). Other kidney lines do not have detectable activity. We have concentrated on LLC cells in studying regulation of proline oxidase. The level of proline oxidase in these cells appears to be corticosteroid inducible. Cells grown in dexamethasone (10^{-8}M) have a threefold increase in proline oxidase activity. The increase requires about 48 hours and is abolished by cycloheximide. The activity of ornithine-ketoacid transaminase, another mitochondrial enzyme, did not change with dexamethasone. These studies were extended to in vivo studies in rats. In adrenalectomized rats, hepatic proline oxidase decreases to about 40% of controls. The falloff occurs by day 2 after adrenalectomy. Administration of repletion glucocorticoids to adrenalectomized rats returned proline oxidase levels to normal. Pharmacologic levels of dexamethasone (20 X repletion dose) increases hepatic proline oxidase activity 2-3 fold over normal controls (4-6 fold over adrenalectomized animals). These studies suggest that proline oxidase can be regulated by glutocorticoids. This regulation may help explain the physiologic and pharmacologic effects of glucocorticoids on proline and collagen metabolism in a variety of tissues.

Mechanism of Action of Growth Hormone

An understanding of the biochemical events accompanying normal growth (as mediated by growth hormone, for example) may be a prerequisite for understanding malignant growth. There is evidence that the anabolic action of growth hormone is mediated by a second protein factor - designated somatomedin. Somatomedin may also be one of the serum factors required for the growth of cells in culture. Work in the laboratory over the past year has continued to be directed toward the purification of a somatomedin like peptide (multiplication stimulating activity or MSA) from the conditioned media of a rat liver cell line that grows without serum. In collaboration with the Diabetes Branch, NIAMDD, highly purified MSA has been shown to compete with human nonsuppressible insulin like activity (NSILA) for binding to a receptor in rat liver membrane. MSA has also been shown to stimulate cell multiplication and ornithine decarboxylase activity in 3T3 cells in culture.

It was previously shown that there was a growth hormone dependent, heat stable, nondialyzable factor in rat serum that stimulates multiplication of chicken embryo fibroblasts in culture demonstrating that chick embryo fibroblasts may be a suitable in vitro system to study the anabolic action of somatomedin. Recent experiments have shown that the serum half life of somatomedin in the rat is growth hormone dependent. When serum from acutely growth hormone treated hypophysectomized rats was injected into hypophysectomized rats the half life was only 15-20 minutes. However, when serum from chronically treated hypophysectomized rats or normal rats is injected into hypophysectomized rats the half life is prolonged to 4-6 hours.

Porphyrin Metabolism

It has previously been shown in this laboratory that a high carbohydrate intake frequently has a major beneficial effect on both the biochemical and clinical manifestations of acute intermittent and variegate porphyria. Previously, initiated studies comparing the effect of glycerol to glucose was continued and it appears that glycerol will be approximately as effective as glucose in these disorders. However, patients seem to often prefer glycerol, stating that they feel much better on the glycerol intake. The dramatic effects of a high carbohydrate intake on the serious neurological manifestations of acute intermittent porphyria were demonstrated in four patients.

In a unique study in a patient with grand mal epilepsy and acute intermittent porphyria, the quantitative effect on the biochemical (and clinical) manifestations of a drug known to be harmful in this disease (Dilantin) was studied. The effects of diet and the interaction of drug and diet were studied. Among the numerous conclusions: 1. drug accounted for at least 50% of the excess urinary porphyrin precursors, 2. high carbohydrate intake could not overcome the detrimental effect of drug and 3. the detrimental effect of drug continued until its blood level was almost down to 0.

In contrast to literature reports of stimulation of protein synthesis in vitro by heme in a number of systems, no effect of heme on hepatic protein synthesis could be demonstrated in vitro.

One of the compounds known to cause experimental porphyria, allylisopropylactemide (AIA), also causes pronounced liver hypertrophy, in studying the mechanism of this hypertrophy, preliminary studies of the effect of AIA on m-RNA and r-RNA synthesis suggest a considerable increase of ribosomal labelling from precursors to occur before any change in m-RNA labelling.

A number of drugs have been tested in animal systems for their effects on porphyrin metabolism. This will serve as a partial basis for evaluating whether they are contraindicated or whether they can be used in certain types of porphyria.

Dr. Kenneth Trabert (Mathematical Biology Branch) has developed a theorem which relates to conditions necessary, but not sufficient for nonlinear differential equations to fulfill in describing continuous oscillatory behavior of a system. This has been applied to enzyme control systems in the study of models of such systems and whether they possess inherent rhythmicity. The predictive value of the theorem has been confirmed in one model by means of computer simulation.

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PHS-NIH
Individual Project Report
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Project Title: Studies of Physiology and Pathophysiology of Plasma Protein Metabolism

Previous Serial Number: SAME

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Warren Strober, M.D., R. Michael Blaese, M.D., K. Robert McIntire, M.D., Samuel Broder, M.D. and Randall S. Krakauer, M.D.

Cooperating Units: Laboratory of Cell Biology, NCI

Man Years:

Total: 6
Professional: 3
Other: 3

Project Description:

Objectives: The objectives of the study were: to determine the normal rates of synthesis, transport and catabolism of the plasma proteins, especially the immunoglobulins, to determine the physiological factors controlling these metabolic parameters and to determine the pathophysiology of disorders of plasma protein metabolism in patients with neoplastic disease and in patients with hereditary disorders of immunoglobulin metabolism. In addition, studies were directed toward determining the sequential steps involved in the differentiation of lymphocyte B-cells to immunoglobulin synthesizing and secreting cells. Major efforts were directed toward defining the role of suppressor lymphocyte T-cells in this maturational process. Finally, studies were directed toward developing techniques for the detection of circulating tumor-related antigens.

Methods Employed: The rates of synthesis, catabolism and distribution of the serum proteins were studied using radioiodinated labelled purified proteins. Double antibody radioimmunoassays were established to detect α-fetoprotein, human chorionic gonadotropin and the immunoglobulins IgG, IgA, IgM, IgD and IgE. As indicated below, a new technique has been developed for the study of the terminal differentiation of B-lymphocytes to immunoglobulin secreting cells.

Major Findings: Sensitive quantitative radioimmunoassays for oncofetal proteins such as alpha-fetoprotein (AFP) and the beta chain of human chorionic gonadotropin (hCG-β) that were established in previous years have been evaluated as tests to assist in the diagnosis of certain forms of malignancy and in the monitoring of the effectiveness of therapy of different forms of cancer. An elevated AFP level (>40ng/ml) was demonstrated in a significant number of the over 1,000 patients with cancer studied including over 70% of the patients with hepatocellular carcinoma or with teratocarcinoma or embryonal cell carcinoma of the testes. In addition, a lesser percentage of patients with entodermal tumors of other organs such as the pancreas, stomach, colon or lung had elevated alpha-fetoprotein levels. In contrast only one of 600 controls or patients with chronic non-hepatic diseases had a serum level over 40 ng/ml. In addition only 1 of 300 patients with tumors of nonentodermal origin such as tumors of the breast, kidney and prostate had an elevated alpha-fetoprotein level.

The radioimmunoassay for alpha-fetoprotein used in conjunction with the assay for hCG-β was of special value in monitoring the effectiveness of therapy of certain forms of malignancy (e.g., embryonal cell carcinoma or teratocarcinoma of the testes) since the products of a few tumor cells were detectable with this assay when they were not detectable when assessed with simple agar diffusion tests. Ninty percent of the over 100 patients with embryonal cell carcinoma or teratocarcinoma of the testes studies had an elevated level of at least one of the marker proteins. Following cancer chemotherapy, there was a reduction of both markers initially. In over 50 percent of the cases, however, although both markers declined, only one of the markers remained elevated and, the remaining marker fell to normal levels. With recurrence of the tumor one of the markers remained at the normal level, whereas the other returned to a very high level. This discordant behavior of these tumor markers suggests that their cells of origin are probably different and underscores the need to follow both markers in evaluating the adequacy of therapy. The importance of following both markers in evaluation of the adequacy of therapy is also supported by the observation that in over 30 percent of the treated patients in the present group there was an elevation of one of the markers at a time following therapy when there was no clinical evidence of residual disease. In each of these cases with an elevation of tumor marker there was a recurrence of the tumor within the subsequent year. Thus, it is our view that these markers should be used in all protocol studies in which patients with testicular tumors are treated, and that the presence of a positive marker should be taken into consideration in determining the future course of the patient's therapy.

Studies of alpha-fetoprotein in patients with benign liver disease were continued. In these studies it was shown that approximately 20% of patients with benign liver disorders have elevated alpha-fetoprotein levels. Concentrations were more frequently elevated in disorders characterized by a prominent parenchimal necrosis, inflammation and extensive liver regeneration. In following patients with active hepatitis there was no positive correlation of an elevated alpha-fetoprotein level in the serum with determinations of the other, hepatic function tests that measure hepatic necrosis, blood flow or protein synthesis. However, in patients with infectious hepatitis the alpha-fetoprotein levels became elevated as the serum SGOT was declining. These observations were taken in conjunction with known features of alpha-fetoprotein synthesis in partially hepatectomized animals supports our view that the alpha-fetoprotein synthesis measures liver regeneration, a function not assessed by other clinical tests.

A significant effort over the past year has been directed toward defining the nature of the events of cellular differentiation, cellular interactions and cellular biosynthesis involved in the specific circulatory and cellular immune responses to antigens. The first group of these studies are directed at defining the factors controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo a sequential maturational process from stem cells originating in the marrow. The first step that does not require antigen but requires a central lymphoid organ involves the maturation of marrow stem cells into B lymphocytes, whereas the second phase which is either antigen or mitogen driven involves terminal differentiation of B lymphocytes into cells that synthesize immunoglobulins and antibodies. The majority of patients with immunodeficiency diseases of man appear to have a defect in the terminal differentiation of B lymphocytes into immunoglobulin synthesizing and secreting cells. We have, therefore, developed a technique for the study of terminal differentiation of such B lymphocytes to define the nature of this critical process and define defects in this process in patients with immunodeficiency disorders. In these studies we have laid special emphasis on the role of regulatory T cells in the control of this maturational event. In the technique utilized, peripheral blood lymphocytes from patients and controls are washed free of serum immunoglobulin and incubated in culture media for a seven day period in the presence of pokeweed mitogen. The IgM, IgA, and IgG molecules synthesized and secreted by these cells into the media are then quantitated using sensitive double antibody radioimmunoassays for each of these immunoglobulin classes.

In normal individuals, the terminal B cell maturational process can be triggered by the pokeweed mitogen. The peripheral blood lymphocytes of normal individuals synthesize 4,900 ng of IgM, 1,260 ng of IgA, and 1,625 ng of IgG per 2 million lymphocytes cultured for seven days in the presence of pokeweed mitogen. Pokeweed mitogen was the only chemical agent to cause a marked and consistent stimulation of immunoglobulin synthesis and maturation and synthesis by B cells. However, a number of physiological factors were shown to inhibit this maturational process. Dibutyryl cyclic AMP or factors which increase intracellular cyclic AMP such as isoproteranol and prostaglandin E_1 prevented B cell maturation and immunoglobulin synthesis. HCG-β and alpha-fetoprotein also inhibited this process. In addition, we have obtained evidence that tissue specific but species non-specific inhibitors of B cell maturation exist (i.e., chalones). In these studies 30-50,000 molecular weight molecules extracted from plasma cytomas of mice with associated hypogammaglobulinemia markedly inhibited the terminal differentiation and immunoglobulin biosynthesis of pokeweed mitogen stimulated human B cells. These materials did not inhibit fibroblast protein synthesis or proliferation. Other materials which inhibit B cell maturation include the mitogens, phytohemagglutinin, staphylococcal filtrate or concanavalin A. That is, when these agents were added to the normal cultures of the lymphocytes in optimal mitogenic doses in the presence of pokeweed mitogen, immunoglobulin synthesis was depressed to 0-16% of normal. Preliminary studies suggest that two mechanisms are of significance in this inhibitory process. The first mechanism is a direct inhibitory action of these soluble mitogens on the B cells. B cells and T cells have equal numbers of receptors for phytohemagglutinin and concanavalin A. Using the present immunoglobulin biosynthesis procedure we have shown that concanavalin A and phytohemagglutinin inhibit the immunoglobulin synthesis of purified B cells. These studies suggest that these agents in soluble form may act to interfere with immunoglobulin production by B cells by interacting directly with the receptors of these mitogens on the B cells leading to an inhibitory signal to these cells. We have, in addition, developed preliminary evidence supporting the view that a subset of the population of T cells can be activated by concanavalin A to non-specifically suppressed antibody production by B-cells.

This technique for the quantitation of in vitro immunoglobulin biosynthesis and secretion was applied to the study of peripheral blood lymphocytes of 22 patients with common variable hypogammaglobulinemia. Four of these patients were able to make small quantities of IgM in vitro when cultured in the presence of fetal calf serum alone. When cultured in their own serum alone or in their own serum plus added fetal calf serum, synthesis of immunoglobulin in this subset of patients was markedly inhibited. In addition, immunoglobulin synthesis by normal cells cultured in the serum of these patients were markedly inhibited. These studies suggest that in this small subset of patients with common variable hypogammaglobulinemia there is a circulating inhibitor of B cell maturation and immunoglobulin synthesis. Lymphocytes of the remaining 18 patients with this disorder studied were not able to make significant quantities of IgA, IgG or IgM in vitro in the presence of absence of pokeweed mitogen.

To determine whether the failure of lymphocyte immunoglobulin synthesis by this second group of patients with hypogammaglobulinemia was due solely to an intrinsic defect of the maturation of their B lymphocytes or whether it could be due to the presence of suppressor cells, the lymphocytes of patients with common variable hypogammaglobulinemia were co-cultured with lymphocytes of normal individuals. When the lymphocytes of those 9 of 13 patients with common variable immunodeficiency studied by this technique were co-cultured with normal lymphocytes in the presence of pokeweed mitogen, the synthesis and secretion of immunoglobulin by the normal lymphocyte was depressed to 0-16% of normal. Incubation with purified T cells from the hypogammaglobulinemic patients resulted in a comparable suppression of the immunoglobulin synthesis by normal lymphocytes. A series of control studies were designed to determine if the cellular suppression process was a specific property of cells from patients with common variable hypogammaglobulinemia. In these studies it was found that co-culture of lymphocytes from unrelated normal individuals did not result in significant inhibition of immunoglobulin synthesis. Thus, the IgM, IgG and IgA synthesized in co-culture by lymphocytes of 20 pairs of unrelated normal donors gave mean values between 93-106% of the expected sum of immunoglobulins synthesized by the two individuals cultured separately. Similarly, incubation of normal lymphocytes with lymphocytes from patients with isolated IgA deficiency or the Sezary syndrome, a T cell leukemia did not inhibit immunoglobulin synthesis. Finally, there was no inhibition of immunoglobulin synthesis by the lymphocytes of 7 normal individuals when they were co-cultured with isolated T cells from unrelated normals. These studies indicate that the ability of cells of one individual to inhibit immunoglobulin biosynthesis by the cells of another is not a nonspecific phenomenon but is a specific feature of the patients with common variable hypogammaglobulinemia. We investigated the capacity of purified peripheral blood B lymphocytes from patients with common variable hypogammaglobulinemia to synthesize immunoglobulin. The unseparated lymphocytes and the purified T cells from these patients studied showed no immunoglobulin synthesis _in vitro_ and depressed the immunoglobulin synthesis of normal lymphocytes by greater than 90%. In contrast, the purified B lymphocytes from these patients did not suppress the immunoglobulin synthesis by normal lymphocytes. In addition, these B cells synthesized normal quantities of IgM when cultured with pokeweed mitogen _in vitro_. These studies suggest that in a significant subset of patients with common variable hypogammaglobulinemia the defect is caused or perpetuated by an abnormality of regulatory suppressor T cells which act to inhibit B cell maturation and antibody synthesis. That is, this subset of patients did not appear to have an intrinsic defect in the maturation of their B lymphocytes, but rather had an abnormal number of abnormally activated set of suppressor T cells.

Patients with thymomas frequently have hypogammaglobulinemia and an increased incidence of infections. We have previously found that 10% of 100 patients studied with thymoma have this associated hypogammaglobulinemia. When the immunoglobulin biosynthesis technique was applied to the study of lymphocytes from two patients with thymoma and hypogammaglobulinemia, a pattern similar to that observed with common variable hypogammaglobulinemia was obtained. That is, the two patients with hypogammaglobulinemia and thymoma studied synthesized less than 100 ng of each class of immunoglobulin. When lymphocytes of these patients were co-cultured with normal lymphocytes and pokeweed mitogen, the synthesis of immunoglobulin by the lymphocytes was depressed by a factor of 66 to 97%. Incubation of normal lymphocytes with purified T cells from the patients with thymoma and hypogammaglobulinemia resulted in a suppression of immunoglobulin synthesis by a factor of 73 to 100%; thus, suppressor T cells may play a role in the pathogenesis of the hypogammaglobulinemia observed in the patients with thymoma and hypogammaglobulinemia studied.

Patients with multiple myeloma have been shown to have a significant reduction in the serum levels of polyclonal immunoglobulins and in the percentage of circulating lymphocytes that have normal surface immunoglobulins. Such patients have been shown to have a reduced capacity to synthesize antibodies in response to antigenic challenge and to have an increased incidence of infections with highly pathogenic encapsulated bacteria. We have applied the in vitro immunoglobulin biosynthesis measurement to the study of peripheral blood lymphocytes from 22 patients with myeloma. The lymphocytes of the patients studied showed profound depression of polyclonal (nonparaprotein class) immunoglobulin production. Thus, the geometric mean polyclonal immunoglobulin synthetic rates were 454 ng for IgM, 321 ng for IgA, and 216 ng for IgG/2 million lymphocytes in culture for 7 days in the presence of pokeweed mitogen. These mean values are approximately 10% of the mean values of normal individuals. Peripheral blood lymphocytes from 3 of the 6 patients with myeloma tested suppressed the polyclonal immunoglobulin synthesis of normal lymphocytes when co-cultured with them. Suppression generally ranged from 75-100%. However, in contrast to the observations in patients with common variable hypogammaglobulinemia and thymoma and hypogammaglobulinemia, this suppression was not observed when purified T lymphocytes of patients with myeloma were co-cultured with lymphocytes of normal individuals. However, in the one patient studied removal of phagocytic mononuclear cells abrogated this suppressor activity. These observations suggest that one mechanism for the humoral immune deficiency observed in patients with myeloma is a block of polyclonal B cell terminal maturation by circulating suppressor cells.

Significance to Biomedical Research: The technique developed for the study of the terminal differentiation of B-cells is of the same value in the study of B-cell maturation as is the lymphocyte blastogenic response to PHA in the study of thymus-dependent lymphocyte (T-cell) maturation. Thus, in vitro techniques are now available to evaluate both arms of specific immune response in man. One can now study the physiological factors regulating this response, one can define defects in the process in patients with immunodeficiency disorders or neoplasia of the B-cell or T-cell lymphocyte system. In addition, such studies can be used to determine the effect of chemotherapeutic or other agents of the terminal differentiation of B-cells in parallel with studies of the effects of these agents on T-cells so that one can define agents which preferentially affect T-cell immunity and those which preferentially effect B-cell immunity. Detection of circulating tumor-related antigens is of significance in the diagnosis of neoplastic disease and is of special relevance in evaluating the activity of the neoplastic disease and determining the efficacy of therapy of these disorders.

Proposed Course: Studies directed toward understanding the pathways and controlling mechanisms of the synthesis, transport, and catabolism of serum proteins will be continued. Special emphasis will be placed on defining the physiological and pathophysiological controlling the sequential development of B cells into immunoglobulin synthesizing cells. Special emphasis will also be placed on the development of new techniques for the detection of circulating tumor-related antigens. These latter studies will include the evaluation of the synthetic rates of catabolism of such tumor-related antigens.

Honors and Awards

Irvin Strasberger Memorial Lecture

John Whitman Memorial Lecture - Lewis Gale Medical Foundation

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Keywords: plasma proteins, transport, catabolism, immunoglobulins, metabolic parameters, neoplastic disease, B-cells, T-cells, alpha-fetoprotein, human chorionic gonadotropin, radioimmunoassay, embryonal cell carcinoma, teratocarcinoma, pokeweed mitogen, thymomas, hypogammaglobulinemia.

Serial No. NCI-Z01-CBD-4003(c)-08-MET
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 20, 1975

Project Title: Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyria

Previous Serial Number: SAME

Principal Investigator: Donald P. Tschudy, M.D.

Other Investigators: C. Richard Magnussen, M.D.

Cooperating Units: Mathematical Biology Branch

Man Years

Total: 4
Professional: 2
Other: 2

Project Description:

Objectives: To study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor-host relationships as well as primary diseases of porphyrin metabolism.

Methods Employed: Quantitative enzyme determinations, chemical determination of porphyrins and porphyrin precursors, isolation, purification and measurement of tissue heme, r-RNA, m-RNA, use of tissue preparations and isotope methods in studying metabolic pathways and computer and mathematical analysis of certain mathematical models.

Major Findings: It has previously been shown in this laboratory that a high carbohydrate intake frequently has a major beneficial effect on both the biochemical and clinical manifestations of acute intermittent and variegate porphyria. Previously initiated studies comparing the effect of glycerol to glucose were continued and it appears that glycerol will be approximately as effective as glucose in these disorders. However, patients seem to often prefer glycerol, stating that they feel much better on the glycerol intake. In collaboration with other investigators treating patients in other parts of the country, the dramatic effects of a high carbohydrate intake on the serious neurological manifestations of acute intermittent porphyria were demonstrated in four patients.

In a unique study in a patient with grand mal epilepsy and acute intermittent porphyria, the quantitative effect on the biochemical (and clinical) manifestations of a drug known to be harmful in this disease (Dilantin) was studied. The effects of diet and the interaction of drug and diet was studied. Among the numerous conclusions: 1) drug accounted for at least 50% of the excess urinary porphyrin precursors, 2) high carbohydrate intake could not overcome the detrimental effect of drug and 3) the detrimental effect of drug continued until its blood level was almost down to 0.

In contrast to literature reports of stimulation of protein synthesis in vitro by heme in a number of systems, no effect of heme on hepatic protein synthesis could be demonstrated in vitro.

One of the compounds known to cause experimental porphyria, allylisopropylacetamide (ALA), also causes pronounced liver hypertrophy. In studying the mechanism of this hypertrophy preliminary studies of its effect on m-RNA and r-RNA synthesis suggest a considerable increase of ribosomal labelling from precursors to occur before any change in m-RNA labelling.

A number of drugs have been tested in animal systems for their effects on porphyrin metabolism. This will serve as a partial basis for evaluating whether they are contraindicated or whether they can be used in certain types of porphyria.

Attempts to quantitatively assay the repressor material of ALA synthetase from tumors have thus far been unsuccessful.

Dr. Kenneth Trabert (Mathematical Biology Branch) has developed a theorem which relates to conditions necessary, but not sufficient for non-linear differential equations to fulfill in describing continuous oscillatory behavior of a system. This has been applied to enzyme control systems in the study of models of such systems and whether they possess inherent rhythmicity. The predictive value of the theorem has been confirmed in one model by means of computer simulation.

Significance to Biomedical Research: Mechanisms of the control of metabolic pathways and the synthesis of enzymes in normal and diseased tissues are being elucidated. Some of the findings have been used for practical application to the diagnosis and treatment of certain types of porphyria. New abnormalities of control mechanisms in tumors and host tissues have been demonstrated and are under current study.

Proposed Course: (A) To continue studies of the factors controlling heme and heme-enzyme synthesis in normal and pathologic tissues. (B) To continue studies of biochemical abnormalities of porphyria in man and experimental animals. (C) To study control mechanisms of heme synthesis in tumors and livers of tumor-bearing hosts.

Honors and Awards

None

Publications

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Keywords: heme biosynthesis, porphyrin metabolism, acute intermittent porphyria, variegate porphyria, high carbohydrate intake, porphyrin precursors.

Serial No. NCI-Z01-CBD-4004(c)-07-MET
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Amino Acid Biosynthesis and Degradation

Previous Serial Number: SAME

Principal Investigator: James M. Phang, M.D.

Other Investigators: S. Peter Nissley, M.D., and Edward Kowaloff, M.D.

Cooperating Units: None

Man Years:

Total: 7
Professional: 3
Others: 4

Project Description:

Objectives: The biosynthesis, degradation and transport of amino acids play an important role in cellular growth and metabolism. The mechanisms by which these processes are regulated in normal and malignant cells are poorly understood. This project emphasizes the regulatory interplay in amino acid metabolism. It is relevant to Objective 3, Approaches 1 and 5 of the National Cancer Program.

Methods Employed: We are primarily using animal cells maintained in long-term tissue culture. Cells can be cloned to insure a genetically homogenous population. Mutants are isolated by standard techniques. A variety of biochemical techniques have been utilized to study amino acid biosynthesis and degradation in cultured cells. Uptake of amino acids may be assessed by using labeled L-amino acids and rates of protein and collagen synthesis can be obtained by incubating cells with L-proline-C^{14} and separation of the radioisotopically incorporated amino acids as proline and hydroxyproline by thin layer chromatography. The enzymes for biosynthesis and degradation of proline are assayed by using appropriate labeled precursors and isolating the products by ion exchange chromatography. Our methods are sensitive enough to measure these enzymes in leucocytes isolated from 10 ml of blood.

Major Findings: In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1-pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase. Hydroxyproline degradation follows a scheme analogous to that for proline. Hydroxyproline is converted first to 3-OH PC and then to γ-OH glutamate.

Phenotypic alterations in P5C reductase. We previously showed that there are two forms of P5C reductase (P5CR) activity in mammalian cells. P5CR$_s$ is sensitive to inhibition by proline (50% inhibition at [proline] = 2×10^{-4}M). P5CR$_i$ on the other hand, is insensitive to inhibition by proline (less than 20% at [proline] = 10^{-3}).

We have shown that:

1. Adult rat liver has predominintly P5CR; whereas fetal liver has 2-3 times the specific activity of P5CR and the enzyme is predominantly P5CR$_s$. The conversion from P5CR$_s$ to P5CR$_i$ progresses with development so that the ratio is about 1:1 at 3-4 weeks of age. 2. Rapidly proliferating cells in tissue culture has only P5CR$_s$. This applies to all epitheloid-fibroblast cells tested. 3. In lymphocytes undergoing phytohemagglutinin-induced blastogenesis, total P5CR activity increases 15 fold and the activity converts from P5CR$_i$ to P5CR$_s$. These observations suggest that accompanying cell proliferation, the activity of P5CR increases but the increased activity is sensitive to feedback inhibition by proline. We are attempting to separate the putative P5CR isozymes on acrylamide gel electrophoresis. Three peaks have been identified. We are presently quantitating the activities in these peaks and characterizing the activities in respect to proline sensitivity.

Type II Hyperprolinemia. We have characterized the genetic defect in type II hyperprolinemia as an absence of P-5-C dehydrogenase (P5CD). Carriers for the defect may be identified by studying P5CD activity in peripheral leucocytes. The transmission of the defect is autosomal recessive. Since patients with the disorder excrete 3-OH-P-5-C as well as P-5-C we considered the possibility that these respective intermediates in the degradation of hydroxyproline and proline may be dehydrogenated by a single enzyme. We enzymatically synthesized and purified radioactively labeled 3-OH-P5C and assayed for conversion of 3-OH-P5C to γ-OH-glutamate in normal and type II hyperprolinemic fibroblasts. The activity in extracts from normal fibroblasts was linear with increasing time and extract protein and was inhibited by P-5-C. In contrast 3-OH-P5C dehydrogenase activity was undetectable in type II hyperprolinemic fibroblasts. This absence of 3-OH-P5C dehydrogenase activity was not due to the presence of an inhibitor since the activity in extracts of normal fibroblasts was not significantly inhibited by the extract from type II hyperprolinemia fibroblasts. These studies suggest that 3-OH-P5C and P5C are oxidized by a single enzyme which is absent in type II hyperprolinemia.

Proline Oxidase. We are studying the enzyme, proline oxidase, which converts proline to P-5-C. The enzyme is undetectable in most cultured cell lines but is found in certain but not all transformed cell lines. The highest levels, however, were found in normal rabbit kidney cells (LLC). Other kidney lines do not have detectable activity. We have used LLC cells in studying regulation of proline oxidase. The level of proline oxidase in these cells appears to be corticosteroid inducible. Cells grown in dexamethasone (10^{-8}M) have a three-fold increase in proline oxidase activity. The increase requires about 48 hours and is abolished by cycloheximide. The activity of ornithine-ketoacid transaminase, another mitochondrial enzyme, did not change with dexamethasone. These studies were extended to in vivo studies in rats. In adrenalectomized rats, hepatic proline oxidase decreases to about 40% of controls. The falloff occurs by day 2 after adrenalectomy. Administration of repletion glucocorticoids to adrenalectomized rats returned proline oxidase levels to normal. Pharmacologic levels of dexamethasone (20 X repletion dose) increases hepatic proline oxidase activity 2-3 fold over normal controls (4-6 fold over adrenalectomized animals). These studies suggest that proline oxidase can be regulated by glucocorticoids. This regulation may help explain the physiologic and pharmacologic effects of glucocorticoids on proline and collagen metabolism in a variety of tissues.

Significance to Biomedical Research: The regulation of amino acids for protein synthesis may play an important role in controlling cell growth. The enzymes involved in amino acid biosynthesis and degradation are affected by genetic, nutritional and hormonal factors. Our studies on the proline metabolic enzymes are contributing to the understanding of these factors. Specifically, genetic defects in the proline metabolic enzymes have elucidated some of the physiologic derangements secondary to a metabolic block. Elucidation of the molecular mechanisms for regulating proline biosynthesis and degradation will lead to an understanding of biochemical regulation in mammalian cells.

Proposed Course: Our studies will emphasize the molecular mechanism producing phenotypic changes in pyrroline-5-carboxylate reductase. Possible mechanisms include 1) selective activation of a gene coding for a different isozyme, 2) post-translational modification of a single gene product and 3) regulated interaction with specific macromolecular ligands. We are performing appropriate experiments to define which of these possibilities are operating in producing P-5-C reductase with varying sensitivities to feedback inhibition by proline.

The presence or absence of proline oxidase in cultured cells is of considerable interest. The physiologic consequences of proline oxidation in cultured cells will be investigated. The ability to use available proline as an energy source may bestow a survival advantage under conditions of relative nutritional deficiency. On the other hand, the degradation of proline may decrease the availability of this amino acid for collagen synthesis. Since proline oxidase may be glucocorticoid-induced, it will be of interest to study those effects in bone and cartilage.

The well-known glucocorticoid effect in decreasing collagen synthesis and bone formation may be related to an effect on proline oxidase. In addition, the presence of proline oxidase only in certain cultured cell lines will allow us to investigate the mechanism by which the activity of this enzyme is regulated. A primary genetic defect or a derangement secondary to abnormal proline or collagen metabolism are intriguing possibilities.

The type II hyperprolinemia fibroblasts continue to be a useful experimental model for answering a number of questions concerning proline metabolism. We will attempt to extend our observations on the inability of these cells to oxidize either P-5-C or 3-OH P-5-C. If we can demonstrate the defect in 3-OH P-5-C dehydrogenation in leukocytes and identify carriers for the defect, we can prove definitively that a single protein is involved in the degradation of 3-PH P-5-C and P-5-C.

Honors and Awards

None

Publications

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Keywords: proline biosynthesis, Δ^1-pyrroline-5-carboxylate reductase, proline oxidase, PC dehydrogenase, isozymes, type II hyperprolinemia, 3-OH-P5C dehydrogenase, corticosteroid inducible.

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1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of the Immune Response in Normal and Pathological States

Previous Serial Number: Same

Principal Investigator: Warren Strober, M.D.

Other Investigators: D.L. Nelson, Z.M. Falchuk, I. Scher, and T.A. Waldmann

Cooperating Units: Division of Gastroenterology, Peter Bent Brigham Hospital, Boston, Mass.; Division of Immunology, National Naval Medical Center, Bethesda, Md.

Man Years:

Total: 6
Professional: 3
Other: 0

Project Description:

Objectives: Studies were directed toward determining the nature and mechanism of circulating and cellular immune responses to antigenic challenges in animals and humans, including patients with abnormal immunologic responses such as gluten-sensitive enteropathy, dermatitis herpetiformis (DH), and inflammatory bowel disease. In addition, studies were directed to determining the mechanism of disease in various immunodeficiency states such as ataxia telangiectasia, hypogammaglobulinemia and intestinal lymphangiectasia. Insight into the cause of the high incidence of reticuloendothelial malignancies associated with these disorders was sought.

Methods Employed· A host of techniques were used to assess immunological function of patients and experimental animals. Circulating B-cells from peripheral blood and tissue were enumerated with fluorescent anti-immunoglobulins. Circulating and tissue T-cells were enumerated by the sheep red blood cell rosette technique. Antibody responses were assessed with microhemagglutination techniques. *In vitro* proliferative responses (micro and macro) were assessed with tritiated thymidine incorporation following exposure to specific and nonspecific mitogens. The presence of mitogen-induced cytotoxic effector cells was assessed with a ^{51}chromium release assay, using red cell and epithelial cell targets. The presence of antibody-mediated cytotoxic effector cells was assessed with ^{51}chromium release assays using red cell and epithelial cell targets in the presence of appropriate antibody. Short-term (1-2 hour) organ culture was used in conjunction with a solid phase immunoabsorption technique to measure local mucosal immunoglobulin synthesis. Long-term (24-48 hour) organ culture was used to develop in vitro models of intestinal disease. Radioimmunoassays in conjunction with lymphocyte culture in the presence of mitogen was used to measure terminal B-cell differentiation. In addition to these specialized techniques, patients with various immunologic disorders were studied with immunoelectrophoresis, radial immunodiffusion, and metabolic turnover techniques for measurement of immunoglobulin synthesis and catabolism.

Gluten-Sensitive Enteropathy: A broad range of studies on the pathogenesis of gluten-sensitive enteropathy were continued. Gluten-sensitive enteropathy (GSE) is a disease of the GI tract associated with malabsorption, villous flattening and increased incidence of neoplasia. It is due to the toxic effect of wheat protein (gluten) on the gastrointestinal mucosa. In previous studies we observed that approximately 80% of patients with GSE carried the HL-A8 histocompatibility antigen. Moreover a similar association between HL-A8 and GI disease occurs in dermatitis herpetiformis, a skin disease associated with a gastrointestinal lesion similar to that seen in GSE. The cause of this association was initially thought to be due to a genetic linkage between the HL-A8 gene (or an alternate histocompatibility gene) and the gene responsible for GSE, the GSE gene. In current studies this concept was explored by analysis of HL-A8 types in the families of some 35 patients with GSE. It was observed that although many siblings and parents of patients with GSE carried the same genotype and/or haplotype as the patient, GSE occurred at a very low frequency in relatives. In one family GSE occurred in 2 siblings, one bearing HL-A8 and one not bearing HL-A8; in another family GSE occurred in a grandmother and 2 of 4 grandchildren, yet neither of the grandchildren bore the HL-A antigens of the grandmother. These family studies indicate that the HL-A8 gene and the GSE gene are not on the same chromosome and the high frequency of HL-A8 in patients with GSE is not due to genetic linkage. On the contrary, these studies indicate that the HL-A8 gene is not merely a marker gene itself but has a direct or indirect effect on the pathologic processes leading to gluten sensitivity.

Additional insight into this problem was obtained from the studies utilizing an in vitro model of GSE previously developed in this laboratory. This model involves the organ culture of jejunal biopsy tissue and measurement of brush border alkaline phosphatase activity during culture as an indication of epithelial cell maturation. As shown previously, alkaline phosphatase activity in tissue from a control patient increases during culture whether or not gluten peptides are present in the culture medium. In contrast, alkaline phosphatase in tissue obtained from patients with GSE increases during culture in the absence of gluten peptides but does not increase during culture in the presence of gluten peptides. Thus, normal tissue matures in culture whereas GSE tissue does not mature in culture when gluten peptides are present.

Using this *in vitro* model of GSE, it was found that tissue from a series of patients with GSE who are HL-A8 positive are inhibited during culture by α-gliadin, a purified wheat protein, or by a cyanogen bromide fragment of α-gliadin. In contrast, tissue from another series of GSE patients who are HL-A8 negative are not inhibited during culture by α-gliadin but were inhibited by the cyanogen bromide breakdown product. These studies indicate that HL-A positive and HL-A8 negative GSE patients are distinguishable in culture at a molecular level. They dovetail with the family studies in that they again indicate that the HL-A8 is not merely a marker gene genetically linked to the GSE gene, but rather that the HL-A8 gene contributes to the disease in a more active way. Since histocompatibility genes control proteins on cell surfaces, it is likely that the HL-A8 gene product contributes to a cell surface receptor responsible for the binding of wheat proteins to cells in the GI mucosa. HL-A8 negative patients have a somewhat different receptor thereby accounting for the different behavior of their tissue in organ culture.

Also using the *in vitro* model of GSE alluded to above, we have initiated studies of the identity of the molecular configuration necessary for toxicity in GSE. In such studies we have identified a molecular fragment having a molecular weight of approximately 20,000 Daltons and a partially characterized aminoacid sequence which shows toxicity *in vitro* for the jejunal tissue obtained from GSE patients. This material is non-toxic to control patients.

The *in vitro* model was also used to further clarify the processes responsible for cellular damage in GSE. In previous studies we have gathered evidence supporting the concept that the pathologic process involved an endogenous mechanism of toxicity which acts through a humoral mediator. In current studies we have shown that *in vitro* gluten peptide toxicity (as indicated by inhibition of alkaline phosphatase increases during culture) is blocked by the presence of steroids in the culture medium. This finding provides additional evidence that gluten peptide toxicity is not a direct toxicity but rather a toxicity that depends upon the mediation of an endogenous mechanism.

Previous studies in this laboratory have shown that patients with GSE respond to the ingestion of dietary gluten with the local production of anti-gluten antibody. These studies, along with the finding of an association between the HL-A8 antigen and GSE, encourages the possibility that the fundamental defect in GSE is the presence of an abnormal immune response gene. If this were so, however, one would expect patients with GSE to exhibit abnormal *in vitro* reactivity to gluten proteins. In current studies we have shown that a variety of gluten protein preparations cultured with lymphocytes obtained from GSE failed to induce proliferative responses. Moreover, parallel attempts to induce lymphokine production (migration inhibition factor) by lymphocytes from GSE patients *in vitro* were also unsuccessful. These experiments are consistent with the concept that gluten proteins are not recognized as immunogens by lymphoid cells unless presented to such cells in a special configuration. The HL-A8 gene product, by participating in the formation of a binding site for gluten in the GI tract, may facilitate the formation of such an immunogenic configuration. In this sense GSE is not due to an abnormal immune response gene but to enhance immunogenecity secondary to a binding phenomena.

During this period a unique immunologic defect of the gastrointestinal tract was identified and extensively characterized. This defect was found in a patient with chronic intestinal moniliasis associated with minimal malabsorption. The patient proved to have normal serum IgA levels, increased numbers of circulating IgA-bearing cells and a normal capacity to produce IgA by peripheral lymphoid cells. Despite these findings the patient had greatly reduced secretory IgA (salivary and jejunal fluid IgA) and a greatly reduced capacity to synthesize IgA in jejunal tissue in vitro. In contrast to patients with IgA deficiency in both the circulation and secretions, this patient had no free secretory component in either saliva or jejunal secretions. These findings indicate that the patient had a normal capacity to elaborate IgA cells but such cells failed to home to (or to differentiate in) the GI mucosa; since the patient lacked the capacity to synthesize secretory piece this molecule may play an important role in the differentiative process of IgA cells.

The study of the immunologic characteristics of the inflammatory bowel disease (ulcerative colitis and regional enteritis) has been continued. These diseases are associated with an increased frequency of gastrointestinal malignancy.

The capacity of lymphocytes of patients with inflammatory bowel disease to react to pokeweed mitogen, lipopolysaccharides and common enterobacterial antigen was studied by measurement of immunoglobulin production in vitro during culture in the presence of these mitogens. The peripheral lymphocytes obtained from patients were cultured after extensive washing and, after 7 days, supernatant was harvested. Ig released into the medium was then measured by a radioimmunoassay technique. We found that cultured cells of inflammatory bowel disease patients produced large amounts of IgA, IgM, and IgG when cultured with pokeweed mitogen. However, neither lipopolysaccharides nor common enterobacterial antigen induced immunoglobulin production beyond that stimulation obtained with the media alone. These studies indicate that a peripheral lymphocyte from inflammatory bowel disease patients do not react selectively and pathologically to either lipopolysaccharides or common enterobacterial antigen with the production of immunoglobulins.

Immune effector mechanisms were studied using nucleated, metabolically active, ^{51}Cr-labeled target cells. It was shown that nude spleen cells could "kill" in antibody-dependent cellular cytotoxicity (ADCC) reaction using EL-4 and anti-EL-4 antibodies but, contrariwise, could not kill EL-4 targets in mitogen-induced cytotoxicity (MICC) reactions. This distinction between ADCC and MICC was not seen when chicken red blood cells were used as target cells. Thus, using metabolically active, nucleated target cells, MICC but not ADCC is T-cell dependent. Similar conclusions are reached if mouse spleen cell populations are purified on sephadex anti-

Fab immunoabsorbent columns. Purified T-cell fractions from such columns were capable of MICC reactions and ADCC reactions. In contrast, B-cell fractions mediate MICC reactions poorly, if at all, but were active in ADCC reactions. It was thought that the ADCC capability of the T-cell fraction was due to Fc-positive Ig-negative cells in the T-cell population, since depletion of a similar T-cell population obtained from human peripheral cells by antigen-antibody plates failed to participate in ADCC; moreover, it was observed that some 30-40% of the T-cell fraction was Fc positive whereas only 3% was Ig positive. These studies now place the identity of cells participating in ADCC and MICC reactions on a firm basis and allows us to study the functional capability of T and B cells of immunologically deficient patients with more certainty: MICC is a T-cell dependent reaction whereas ADCC is a T-cell independent reaction.

The cellular definition of cytotoxicity reactions indicated above have been applied to the study of patients with intestinal lymphangiectasia. In the past we have shown that such patients have a protein-losing enteropathy associated with loss of lymphocytes into the gastrointestinal tract. The lymphocyte loss leads to anergy and decreased capacity to proliferate in vitro in the presence of the T cell mitogens. In current studies it was shown that peripheral blood lymphocyte populations contained reduced numbers of E-rosette forming cells (T-cells) and increased numbers of cells with surface immunoglobulins (B-cells). In contrast, analysis of pleural effusion lymphocyte population revealed increased numbers of E-rosette-forming cells relative to the number found in the patients' peripheral blood and equal numbers of these rosette-forming cells relative to the number found in normal peripheral blood. Concomittently, the percentage of pleural effusion lymphocytes bearing cell surface immunoglobulins was less than that in the patient's peripheral blood and equal to the percentage of B-cells in normal peripheral blood. These studies of surface markers complement studies of the cytotoxic capacity of lymphocyte populations derived from peripheral blood and pleural fluid. It was found that mitogen-induced cytotoxicity of the pleural fluid was greatly enhanced compared to that in peripheral blood. This is understandable in view of the increased numbers of T cells in the pleural fluid cell population. In general, these studies indicate that patients with intestinal lymphangiectasia have a selective loss of a T-cell population capable of proliferative responses in vitro and mitogen induced cellular cytotoxicity reactions. The fact that the cells capable of such in vitro reactivity is present in the pleural fluid indicated that there is no loss in the capacity to synthesize such cells but rather that the defect is on the catabolic or loss side of the metabolic cycle.

Significance to Biomedical Research: Studies of gluten-sensitivity have provided important new insights into the nature of immune responses. This disease is the only disease in which a high association with a histocompatibility antigen has been found and in which the toxic agent, in this case, gluten protein, is known. This disease is therefore an important model for the study of the association of histocompatibility antigens and disease. In the current group of studies we have learned that this association is not trivial or happenstance. The HL-A8 gene is not merely a marker gene but actually takes part in the disease process. Evidence has been accumulated that the role of the HL-A8 gene and its product involves the synthesis of a cell surface receptor which is instrumental in the formation of an immunogenic molecular configuration for gluten. In this way a food protein which does not normally evoke an immune reaction in the GI tract, becomes immunogenic and toxic.

Studies of the immunology of the GI tract, particularly the organized gut associated lymphoid tissue in Peyer's patches, may lead to an increased insight into the mechanism whereby the GI tract establishes an equilibrium with the normal bacterial flora of the bowel. As such, these studies can lead to an understanding of the relationship between normal cell proliferative processes in the GI tract and the abnormal cell proliferative processes observed in GI neoplasms.

It is well-known that patients with gluten-sensitive enteropathy, and inflammatory bowel disease as well as patients with immunodeficiency are subject to a greatly increased risk of neoplasia. A variety of evidence point to the immunologic system as an important bulwark against the development of neoplasia. Studies of the diseases characterized by immunologic system acts to prevent neoplasia as well as how aberrant immune responses may predispose to neoplasia.

Proposed Course: Investigation of the role of immunologic factors in gastrointestinal disease will be pursued. The in vitro model of gluten-sensitive enteropathy will be used to identify the precise molecular configuration necessary for toxicity. In this regard, gluten obtained from mutant wheat strains lacking certain gliadin components will be tested in the organ culture system for toxic potential; such studies could conceivably lead to the development of non-toxic wheats. Also using the in vitro model, differences and similarities between the intestinal lesion of dermatitis herpetiformis and gluten-sensitive enteropathy will be explored. These studies will help understand the role of the HL-A8 gene in the etiology of these diseases.

In the area of inflammatory bowel disease, continued investigation of the immunologic reactivity of both mucosal and peripheral lymphocytes to gut constituents will be carried out. In this regard the study of lymphocyte Ig production in vitro in response to lipopolysaccharides and enterobacterial common antigen will be pursued using purified T-cell and B-cell populations as well as lymphoid populations obtained from normal and diseased gut.

Fundamental studies of gut immunology will be pursued using the model of in vitro terminal B-cell differentiation. The organized lymphoid tissue of the GI tract of experimental animals will be studied in culture for their potential to differentiate into IgA producing cells. In this connection the effects of co-culture with T-cells, allogeneic cells and with secretory piece will be investigated.

Honors and Awards

None

Publications

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Keywords: immune response, gluten-sensitive enteropathy, dermatitis herpetiformis, inflammatory bowel disease, tritiated thymidine, mitogen-induced, cytotoxic, gliadin, alkaline phosphatase, lipopolysaccharides, target cells, E-rosette-forming, histocompatibility antigen

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1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of the Development and Function of Humoral and Cellular Immune Mechanisms of Host Defense

Previous Serial Number: SAME

Principal Investigator: R. Michael Blaese, M.D.

Other Investigators: Thomas A. Waldmann, M.D., Warren Strober, M.D., J. Wunderlich, M.D., A.V. Muchmore, M.D., R. Young, M.D., H. Kirchner, M.D., D.L. Nelson, M.D., K. Takemoto, M.D., and D. Poplack, M.D.

Cooperating Units: Immunology Branch, NCI, and Medicine Branch, NCI, Pediatric Oncology Branch, NCI, and Laboratory Viral Diseases, NIAID.

Man Years:

Total: 4
Professional: 2
Other: 2

Project Description:

Objectives: The objectives of this study were to determine mechanisms important to the development and function of both humoral and cellular immune response in animals and man; to determine the nature of the deficiency in immune function characterizing such disease states as the Wiskott-Aldrich syndrome, intestinal lymphangiectasia and advanced malignancy; and, to develop approaches to prophylaxis and/or therapy of the infectious and neoplastic disease frequently associated with defective immune processes. This work is directly related to Objective 2, Approach 1 of the National Cancer Plan.

Methods Employed: Antibody responses of experimental animals and man were determined after immunization with bacterial vaccines such as brucella abortus, S. typhosa, and pertussis; with particulate cellular antigens such as sheep, burro, and chicken RBC's; with purified protein antigens such as KLH, diphtheria and tetanus toxoids, guinea pig-γ-globulin and bovine γ-globulin; with purified polysaccharide antigens such as pneumococcal polysaccharides types I, II, III, and the V_1 antigen of E. coli. The antibodies were detected by standard techniques or by newly developed micro methods of bacterial agglutination, red cell hemolysis, and passive hemagglutination using $CrCl_3$ as an agent to couple the antigens to a carrier RBC surface. In addition, modifications of the Farr antigen binding assay were used.

Cellular immune responses were measured by delayed hypersensitivity skin tests, contact sensitivity to DNCB, and skin allografts. Lymphocyte transformation in vitro was carried out utilizing non-specific mitogens such as PHA, PWM and staph filtrate; specific antigens such as SLO, SKSD, diphtheria and tetanus toxoids, KLH and candida albicans; and allogeneic cells in mixed leukocyte culture. Lymphocyte function in vitro was also measured by production of mediators of cellular immunity such as lymphotoxin, MIF, and lymphocyte derived chemotactic factor for monocytes (LDCF) and by cytotoxicity reactions with antibody sensitized target cells or with non-specific mitogen activated effector lymphocytes.

Studies of the characteristics of various cell surface receptors on immunocompetent lymphocytes and monocytes-macrophages utilized autoradiography, immunofluorescence microscopy, and cellular rosette formation.

Major Findings: Study of patients with immunodeficiency disease and a high incidence of malignancy was continued with the ultimate goal of correction of the immunologic defect in these patients and gaining insights into the role of immune processes in prevention and control of malignant disease.

The Wiskott-Aldrich syndrome an x-linked immunodeficiency disorder, has a very high incidence of infections with all classes of microorganisms and, in addition, the highest rate of malignant disease of any of the immunodeficiency diseases. Intensive study of these patients has demonstrated pronounced defects in both humoral and cellular immunity characterized by clinical anergy and defective antibody production. In spite of these pronounced clinical immune defects, our studies have demonstrated that much of the effector arm of their immune system is intact. They have normal numbers of circulating T and B-lymphocytes as detected by immunofluorescence or microcytotoxicity assays with specific antisera. They produce immunoglobulins at normal or elevated rates. Their lymphocytes have normal proliferative responses in vitro to non-specific mitogens and they produce lymphokines such as macrophage migration inhibitory factor (MIF), lymphotoxin and chemotactic factor for monocytes normally. In addition, the killer function of their B lymphocytes is normal as detected by in vitro antibody directed lymphocyte mediated cytotoxicity, and their T lymphocyte killer cells are normal as tested in PHA activated killing of ^{51}Cr-labelled target cells in vitro. In spite of these normal in vitro tests these patients lack adequate specific humoral and cellular immune responses both in vitro and in vivo which supports our view that the immunodeficiency of this disorder is the result of a defect in afferent limb of immunity, perhaps a defect in antigen recognition or processing or representing defective control of their immune responses. Defective control of immunoresponsiveness is also suggested by the observation that many of these patients have developed transient monoclonal gammopathy. In addition, the unstimulated lymphocytes from these patients in vitro were found to spontaneously produce as much of a chemotactic factor for monocytes as do lymphocytes from normal individuals only after antigen or mitogen stimulation. This high level of unstimulated chemotactic factor production apparently contributes to the anergy in these patients by producing high circulating levels of chemotactic factor which tends to abolish any chemotactic gradient produced by a specific immune response and also by deactivating the chemotactic responsiveness of the monocytes from these patients.

A most striking feature of the WAS is the very high incidence of lymphoreticular malignancy found in these patients, Six of the 13 patients studied at NIH since 1966 have developed malignancy. In a very provocative study in collaboration with Drs. K.K. Takemoto and A.S. Rabson, we have isolated a papovavirus from a reticulum cell sarcoma involving the brain of one of our patients. Serum from this patient was studied for antibody to the virus and about 14 months prior to the clinical appearance of the tumor the patient developed a striking antibody titer to this virus. Following the isolation of this papovavirus from the child's brain tumor, the virus was also isolated from his urine on each of 3 attempts. The isolation of a virus belonging to the papova group, which are well-known for their oncogenic properties, from a human tumor raises intriguing questions in regard to the possible role of these viruses in human cancer. Although this single observation certainly does not prove an etiologic relationship between this virus and cancer, it does provide a strong stimulus to further investigation of it association with malignancy, particularly that occurring in immunodeficient individuals. In this regard, a similar papovavirus has been isolated from the urine of 4 of 4 additional WAS patients, one of whom also had a lymphoma. Virus was not isolated from 14 patients with other immunodeficiency diseases.

Studies of many aspects of cell-mediated immunity were continued in a variety of basic model systems and several of these systems were extended to study the state of immune competence of patients with neoplastic disease and/or immune deficiency disease. Cellular immune function in vitro has been assessed by four fundamentally different techniques: 1. proliferative response of lymphocytes following stimulation with mitogens, specific antigens, and allogeneic cells; 2. the production of biologically active soluble mediators (lymphokines) secreted by activated lymphocytes; 3. the cytotoxic killer cell activity of both immune and non-immune lymphoid cells and macrophages; and 4. the in vitro responsiveness of mononuclear leukocytes (monocytes and macrophages) to various lymphokines and other biological mediators such as activated serum complement components.

One leukocyte function receiving intensive investigation in many laboratories is the cytotoxic effector capacity or killer activity by which lymphoid cells destroy various tumor cells and other tissue targets. Studies of the identity, characteristics and mechanisms of cells involved in these reactions have been a major effort of this section during the past year. To investigate the basic mechanisms of cytotoxic reaction, two model systems (ADCC or antibody-dependent cellular cytotoxocity and MDCC or mitogen-dependent cellular cytotoxicity) have been employed. ADCC is a cytotoxic reaction in which target cells coated with specific antibody are killed by nonimmune effector lymphoid cells. The identity of the effector cell-mediating ADCC has been the subject of considerable controversy with little agreement between the various laboratories involved in these studies. We have found that many cell types possess the capacity to kill various antibody coated target cells. This activity depending upon the nature of the target cell under attack and the type of antibody utilized in the system. When human HLA-7 bearing lymphocytes are utilized as targets with a human anti-HLA-7 antibody, the effector cell leading to target cell destruction is apparently a B-lymphocyte in man. T-cells, polymorphonuclear leukocytes nor monocytes are capable of mediating cytotoxicity in this particular system. By contrast, if human group B red blood cells coated with human anti-B antibody are utilized, only monocytes and macrophages are capable of effecting these cytotoxic reactions, whereas T-cells, B-cells and polymorphonuclear leukocytes are not. Finally, in yet another system, if chicken red blood cells are used as targets with a rabbit hyperimmune anti-chicken red cell antibody, B-cells, macrophages and monocytes, polymorphonuclear leukocytes and even non-lymphoid cell lines and tumor cells such as rat embryo fibroblasts or the P815Y mouse mastocytoma cell line are capable of initiating the killer reaction. Thus, antibody dependent cell-mediated cytotoxicity can be a reaction mediated by a small well-defined group of effector cells or by a large number of different forms of effector cells including even non-lymphoid cells. It is particularly noteworthy that each of the effector cells active in antibody cellular cytotoxicity have a receptor for the activated Fc portion of IgG. A number of tumor cells have also been described which contain such Fc receptors and these studies have demonstrated that some of these tumor cells may themselves become cytotoxic effectors in this *in vitro* system.

Studies of patients with neoplastic and immune deficiency disease employing assays for antibody-dependent cellular cytotoxicity have revealed a number of interesting characteristics. As might be expected, patients with aplastic anemia have defective killing of human red blood cell targets in one ADCC system, since the killing in this system is dependent on macrophage function. However, these patients have normal cytotoxic activity when assayed in ADCC systems utilizing B-lymphocytes as the effector. Patients with agammaglobulinemia who lack circulating B-cells have defects in that in the form of ADCC requiring B-lymphocytes as a killer but have normal antibody dependent cellular cytotoxicity reactions in systems that utilize monocytes, polymorphonuclear leukocytes or other effector cell populations. Patients with intestinal lymphangiectasia, a protein-losing gastroenteropathy associated with the generalized lymphatic abnormalities and lymphocytopenia, were found to have deficient T-lymphocyte proliferative responses in their peripheral blood. The ADCC responsiveness of peripheral blood lymphocytes however, was augmented compared with normal controls suggesting that the cell population lost by patients with intestinal lymphangiectasia does not include those cells participating in this form of cytotoxicity. Conversely, chylous effusion lymphocytes obtained from patients with this disease, cells which enter the effusions directly with lymph were found to have a normal proliferative response and a defective cytotoxic reactivity in the ADCC system for B-lymphocyte killing.

Mitogen activated cellular cytotoxicity is another model system for cell-mediated target cell killing which utilizes a mixture of effector cells, target cells and various plant lectins to develop cytotoxicity. Lectins such as phytohemagglutinin, concanavalin A and pokeweed mitogen are effective in inducing the killer cell reaction to a variety of target cells. Here again we found that these assays are very dependent upon the type of target cell studied and specific lectin employed. It was found that the lectin provides a specific link between the effector cell and target cell so that the cytotoxic reaction can proceed. The lectin provides a certain degree of specificity in these reactions for both the effector and target cell must have a receptor for the lectin in order for the linking to proceed normally. Thus, concanavalin A will induce mammalian effector cells to kill burro erythrocytes which have a receptor for concanavalin A but will not induce the same effector population to kill human erythrocytes which lack a receptor for concanavalin A. Conversely, pokeweed mitogen induced killing of human erythrocytes but not of burro erythrocyte target cells. As in the system of antibody dependent cellular cytotoxicity there is specificity as to the killer cells which are effective for different classes of target cells. Metabolically active target cells such as the P815Y mouse mastocytoma tumor cell are killed only in the presence of a mixture of T-lymphocytes and mitogen. Thus, cells from athymic nude mouse are unable to kill this target cell in the presence of phytohemagglutinin. However, if chicken erythrocytes are employed as the target cell, athymic nude mouse spleen cells are very effective in inducing the killer reaction of these targets in the presence of PHA. In fact, erythrocyte target cells are killed by T-cells, B-cells, macrophages and such non-lymphoid cells as cultured mouse L-cells, rat embryo fibroblasts and the P815Y mastocytoma tumor cell line. Thus, similar to the heterogeneity of effector function observed in antibody-dependent cellular cytotoxicity, mitogen-dependent cellular cytotoxicity also may reveal the effector potential wide variety of both lymphoid and both non-lymphoid cells.

Another major area of investigation by the cellular immunology section has involved various mechanisms of immunoregulation. One of the most striking examples of physiologic immunoregulation occurs during pregnancy as demonstrated by the absence of immunologic attack by maternal cells on the histoincompatible placenta and fetus. Among several cooperating factors contributing to this lack of immune rejection, we have found that human chorionic gonadotropin (HCG), a glycoprotein hormone produced in vast quantities by the syncitiotrophoblast of the placenta, is a potent immunosuppressive compound. HCG in concentrations found at the junction of the placenta and uterine vessels inhibited proliferative and cytotoxic effector cell activity of human peripheral blood lymphocytes. The most profound inhibitory activity on proliferative responses was noted in specific antigen induced lymphocyte responses and in mixed lymphocyte reactivity, while non-specific mitogen induced reactivity was inhibited by higher HCG concentrations. In addition, the cytotoxicity effector function of lymphoid and non-lymphoid cells was also blocked by HCG in ADCC and MDCC assays. The immunosuppressive effect of HCG was completely reversible so that cells washed free of the hormone regained normal functional capacity. Thus, HCG possesses many of the properties of an ideal immunosuppressive agent for pregnancy. It works most efficiently in inhibiting specific antigen induced immune responses; it is an efficient immunosuppressive only in concentrations found at the site of maximum desired effect, (the placenta-uterine interface); its immunosuppressive effects are completely reversible so that generalized maternal immunocompetence is not impaired; and it suppresses both the recognitive-proliferative phase and also the cytotoxic effector phase of cellular immunity.

Another form of immunoregulation with potentially profound effects on both normal host immune responses and in the pathogenesis of disease, the suppressor T-cell, was studied in an experimental model system of agammaglobulinemia in chickens. Avian species have a central lymphoid organ for the humoral immune system, the Bursa of Fabricus. Removal of the Bursa in the immediate post-hatching period with the addition of sublethal irradiation results in the development of agammaglobulinemia when the birds mature. This immune deficient state is characterized by total deletion of the humoral immune system with absence of immunoglobulins in serum and secretions, absence of plasma cells and germinal centers in lymphoid tissue and absence of B-lymphocytes in the lymphoid organs and circulation. Functions of the thymic dependent immune system remain intact in these animals. During experiments designed to reconstitute humoral immune responsiveness in such agammaglobulinemic birds by transplantation of bone marrow from normal syngeneic donors, it was observed that immunoglobulin production could not be restored in the transplanted agammaglobulinemic birds even though cytogenetic analysis revealed successful engraftment by the cells from the normal donor. The converse experiment resulted in even more curious results. When normal birds were grafted with thymus or bone marrow cells obtained from agammaglobulinemic donors, the recipient normal birds developed agammaglobulinemia. This "infectious agammaglobulinemia" differs from that induced by bursectomy in the new-hatched period in several critical respects. Although these birds lack detectable immunoglobulin and antibody responsiveness, they continue to have "B" lymphocytes in their spleens and peripheral blood as determined by the presence of surface bound immunoglobulin detected by immunofluorescence. In addition, the Bursa of Fabricus remained present in these birds and their lymphoid tissues contained some lymphoid follicles and occasional germinal centers. Thus, the immune deficit in these birds closely resembles that seen in the majority of our patients with the disorder known as common variable hypogammaglobulinemia. In both the human disease and in this "infectious agammaglobulinemia" of chickens, excessive activity of a population of suppressor T-lymphocytes can be implicated in the pathogenesis of the humoral immunodeficiency.

Patients with Hodgkin's and non-Hodgkin's lymphoma have a variable degree of in vivo and in vitro immunodeficiency. Patients with the most advanced disease were found to have dininished numbers of circulating T-lymphocytes and decreased in vitro proliferative responses, particularly when stimulated with specific antigens in vitro. Killer lymphocyte function in many of these patients was also defective while their ability to produce a lymphokine chemotactic for monocytes was generally in tact. The responsiveness of monocytes from these patients to chemotactic factors was also frequently abnormal. During the course of therapy, those patients having a beneficial response showed remarkable recovery of in vitro cellular immune function, while those patients who were treatment failures also failed to develop improved cellular immune function in vitro. It thus appears that study of in vitro lymphocyte function may provide a useful prognostic index for the immediate prospects of remission in these lymphoid malignancies, although only longer periods of follow-up will indicate the long-term prognostic implications of these observations.

Studies of various factors influencing the development of immunocompetence in the neonatal period were continued. We have previously shown that neonatal rats acquire immune responsiveness to different antigens in a distinct sequence rather than simultaneous in early postnatal life. The apparent sequential maturation of immune competence was found not to be dependent upon stepwise differentiation of specific antigen reactive cells, but rather upon the maturation of components of the afferent limb of immunity. Macrophages play an essential role in the initiation of immune responses to many antigens, and it was demonstrated that immaturity of this system of cells accounted for much of the apparent immune deficiency in the neonate. Thus, when mature adult macrophages were given to the neonate, they produced antibody to a variety of antigens to which they were ordinarily unresponsive. It was also found that a variety of materials given to the neonates would result in accelerated maturation of their own macrophage population, and result in earlier immunocompetence. Thus, the adjuvant properties of such agents as endotoxin, BCG, alum, pertussis vaccine, silica, and poly A.U. were shown to function by macrophage activation. We have also shown that activation of the macrophage system of the neonate not only allowed antibody formation in otherwise unresponsive neonates, but also was effective in preventing the induction of specific immunological tolerance by high doses of antigen given at birth. In preliminary studies this finding was utilized to prevent tolerance to the vertically transmitted mammary tumor virus in C3H mice and this non-specific immunoprophylaxis subsequently resulted in resistance of these mice to mammary tumor transplantation. Macrophage activation in neonatal C57 BL/6 mice, however, did not affect subsequent development of progressive tumors caused by neonatal infection with M-MSV.

Further studies of role of afferent limb were carried out to determine its effect on the cellular immune system. Adult macrophages and macrophage activating substances were found to enhance the ability of spleen cells from neonatal rats to induce a graft versus host reaction in F_1 hybrid recipients. Macrophage activation also promoted both the development of delayed type hypersensitivity and its expression by skin testing. Macrophage activation also significantly enhanced expression of cellular immunity to RNA virus associated tumor antigens in mice.

Significance to Biomedical Research: The present studies extend our understanding of the diverse, yet interrelated mechanisms contributing to the development and normal functioning of immune system in animals and man. They indicate that disorders of immune function may be related to the absence of malfunction of such central lymphoid organs as the thymus or Bursa of Fabricius or to immaturity of such auxillary systems as the macrophage required for the processing of antigens in the initiation of an immune response. They also indicate that the macrophage plays multiple roles in a normal immune response, being both important in the initiation or afferent limb of such responses and also as the ultimate effector cell in many immune deficiency and advanced malignancy have extended our understanding of the nature of the reactions produced by different classes of lymphocytes and the observation that some of these functions may remain intact in severely anergic individuals may provide us with a mechanism of providing effective immunotherapy for malignancy occurring even in severely immune deficient patients. Similarly, the observation that the monocytes and macrophages are abnormal in some patients with cancer or immunodeficiency and that this defect may be corrected by transfer factor treatment offers another approach to utilize immune processes in therapy of disease.

Proposed Course: Continuing studies directed toward understanding the normal pathways involved in the development and function of the immune system and application of this new knowledge in approaches to the prevention and therapy of malignant disease.

Honors and Awards

Elected to Membership in The Society for Pediatric Research

Publications

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Kirchner, H., Muchmore, A.V., Chused, T.M., Holden, H.T. and Herberman, R.B.: Inhibition of proliferation of lymphoma cells and T lymphocytes by suppressor cells from spleens of tumor-bearing mice. *J. Immunol*. *114*: part 1, 206-210, 1975.

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Keywords: humoral and cellular immunity, Wiskott-Aldrich syndrome, intestinal lymphangiectasia, neoplastic disorders, brucella abortus, keyhole limpet hemocyanin, transfer factor, delayed hypersensitivity.

Serial No. NCI-Z01-CBD-4016(c)-02-MET
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism of the Anabolic Action of Growth Hormone

Previous Serial Number: SAME

Principal Investigator: S. Peter Nissley, M.D.

Other Investigators: Kenneth L. Cohen, M.D.

Cooperating Units: Diabetes Branch, NIAMDD

Man Years:

Total: 3.5
Professional: 2
Other: 1.5

Project Description:

Objectives: The understanding of normal growth may be prerequisite to understanding malignant growth. One experimental model for the study of normal growth has been the growth hormone treated hypophysectomized animal. It has been proposed that the anabolic action of growth hormone is mediated by a second circulating protein designated somatomedin. The object of this project is to purify somatomedin, develop an in vitro tissue culture system responsive to somatomedin and study the biochemical events accompanying growth in the system.

Methods Employed: Somatomedin is being purified from the conditioned medium of rat liver cells in culture. This rat liver cell line grows in the absence of serum and produces a growth factor identical to or closely related to somatomedin (multiplication stimulating activity or MSA). Serum starved cells in culture are being utilized to study the action of purified MSA. DNA synthesis is being measured by thymidine incorporation into DNA, flow microfluorometry and cell multiplication. The thymidine incorporation assay in chick embryo fibroblasts is being utilized to measure the serum half-life of somatomedin in hypophysectomized rats.

Major Findings: It was previously shown that there is a growth hormone dependent, heat stable, non-dialysable factor in rat serum that stimulated the multiplication of chick embryo fibroblasts in culture, thus demonstrating that chick embryo fibroblasts may be a suitable in vitro system to study the mechanisms of action of somatomedin. These experiments have now been extended utilizing serum from growth hormone deficient patients before and after growth hormone treatment. Serum from growth hormone treated hypopituitary patients stimulates thymidine incorporation into DNA in chick embryo fibroblasts at 2-3 fold the level seen with sera from hypopituitary patients before growth hormone treatment.

Utilizing the thymidine incorporation assay in chick embryo fibroblasts to measure somatomedin it has been shown that the serum half life of somatomedin is growth hormone dependent. When serum from hypophysectomized rats acutely treated with growth hormone was injected into hypophysectomized recipient rats, the serum half life was only 15-20 minutes. However, when normal rat serum or serum from hypophysectomized rats chronically treated with growth hormone was injected into recipient hypophysectomized rats, the half life was 4-6 hours. The half life of somatomedin in normal rat serum can be shortened to 15-20 minutes by heat treatment under acid conditions or by passing normal serum over sephadex G-50 in 1N acetic acid. These experiments are compatible with the induction of a somatomedin binding protein by chronic growth hormone treatment. The binding protein alters the half life of somatomedin.

In collaboration with the Diabetes Branch, NIAMDD, highly purified MSA has been shown to compete with non-suppressible insulin-like activity (NSILA) for binding to a liver membrane receptor.

Highly purified MSA has also been shown to stimulate DNA synthesis and multiplication of 3T3 cells in culture. MSA also causes a five-fold increase in ornithine decarboxylase activity in 3T3 cells. An increase in ornithine decarboxylase activity has been associated with many examples of growth stimulation in vivo and in vitro.

Significance to Biomedical Research: The understanding of the biochemical events accompanying normal growth should provide a groundwork for understanding the aberations in growth accompanying malignancy. Also, further delineation of the hormonally dependent growth factors necessary for the growth of cells in culture is of general importance in tissue culture, a widely utilized technique in cancer research. Specifically this research supports objective 3, approaches 1 and 2 and objective 4, approach 1 of the National Cancer Plan.

Proposed Course: Skin fibroblasts have been cultured from two growth hormone deficient patients and will be grown in these same patient's sera taken before and after growth hormone treatment to determine whether or not the multiplication of human skin fibroblasts in culture is under growth hormone control. Utilizing the liver membrane radioreceptor assay for MSA, MSA levels will be measured in normal, hypophysectomized, growth hormone treated hypophysectomized and GH secreting tumor bearing rats to determine whether or not MSA is growth hormone dependent. A radioimmunoassay for MSA is also being developed to confirm the results of the radioreceptor assay. The anabolic action of MSA on cells in culture will continue to be studied as an in vitro model for growth.

Honors and Awards

None

Publications

Cohen, K.L., Short, P.A., and Nissley, S.P.: Growth hormone-dependent serum stimulation of DNA synthesis in chick embryo fibroblasts in culture. Endocrinology 96: 193, 1975

Keywords: Growth, growth hormone, somatomedin, tissue culture, multiplication stimulating activity, DNA synthesis, thymidine incorporation, chick embryo fibroblasts, serum half life of somatomedin, growth hormone deficient patients, non-suppressible insulin-like activity, liver membrane receptor, 3T3 cells in culture, ornithine decarboxylase.

SUMMARY REPORT
LABORATORY OF PATHOLOGY
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE
July 1, 1974 to June 30, 1975

The diagnostic and research activities of the staff of the Laboratory of Pathology are listed by Sections. The research accomplishments are especially impressive when one considers that the majority of the staff participate to some extent in the diagnostic services for Clinical Center patients. In fact, because of changes in professional personnel, including resignations and retirement of some staff members, and because of additional administrative responsibilities of others, the overall diagnostic workload of several members of the staff has increased appreciably during the past year. The assignment of Dr. Jose Costa to the Surgical Pathology Section, the employment of Dr. Timothy J. Triche in Ultrastructural Pathology and Dr. Nathaniel A. Young in Viral Oncology and Molecular Pathology helped a great deal to alleviate this situation. It is by offering opportunities and facilities for laboratory research that we have been able to recruit and retain an outstanding group of anatomical pathologists who have given vital support to the clinical programs of NCI as well as all the other Institutes with patients in the Clinical Center.

The research programs of the Laboratory have involved:

A. Hematopathology and Immunopathology
B. Viral Oncology and Cell Biology
C. Comparative Pathology and Carcinogenesis
D. Development and use of Automated Equipment for Cytology Diagnosis
E. Clinico-pathologic Studies on a Variety of Human Diseases

Some of the significant research and clinico-pathologic accomplishments in which the staff of the Laboratory participated, either as principal investigators or as collaborators include:

1. Isolation and successful culture of a human papovavirus from a human brain tumor.

2. Characterization of surface receptor sites and immunoglobulin production by normal and neoplastic cells from patients with a wide range of malignant lymphomas and leukemias.

3. Demonstration that fiber size is critical not only for asbestos carcinogenesis but also for other particulate material including silicon carbide and certain fibrous talcs.

4. Demonstration of an association between H. varicellae in dorsal root ganglia and in herpetic skin lesions (shingles) in a patient with malignant lymphoma.

5. Determination of the Coulter volume distribution of cells from normal and abnormal cervico-vaginal specimens.

6. Use of flow analytic techniques to measure efficiently and accurately the blastogenic response of lymphocytes to various antigens.

7. Evaluation by EM of hepatic fibrosis in workers exposed to vinyl chloride.

8. Characterization of cell-mediated immunity in patients with retinal pigment degenerations and in patients with retinoblastomas.

Many members of the staff, in addition to their laboratory and service work, serve on numerous national and international committees, editorial boards, etc. Some of the principal activities of this sort are: Dr. Powell, member of International Association for Study of Lung Cancer; Dr. Thomas, WHO Collaborating Center for Classification of Uterine Tumors, WHO Committee to Develop International Classification of Disease - Oncology (ICD-0), USA Mycosis Fungoides Panel, and Council member of International Academy of Pathology; Dr. Herman, Chairman of Committee on Cytology Automation; Dr. Berard, Pathology Editor of the Journal of RES, Associate Editor of Laboratory Medicine and member of USA Lymphoma Panel, Co-Director of Long Course of International Academy of Pathology; Drs. Berard, Jaffe and Braylan, faculty of Tutorial on Neoplastic Hematology held in Chicago and in Paris; Dr. Henson, Short Course on Pathology of Virus Infections, IAP; Drs. Thomas, Rabson and staff, lecture series in pathology to Georgetown University medical students; Dr. Dawe, Project Officer for Registry of Tumors in Lower Animals (Smithsonian Institution); Dr. Henson, member of College of American Pathology Committee on Nomenclature; Dr. Chu, faculty of Aspiration Cytology Workshop, American Society of Cytology.

Dr. Alan Rabson, Deputy Chief of the Laboratory of Pathology and Head of Viral Oncology and Molecular Pathology Section, was appointed Director of the Division of Cancer Biology and Diagnosis early in 1975. Dr. Nathaniel Young who had joined the VOMP section in July 1974 was appointed Head of the section following Dr. Rabson's reassignment. It is planned to reassign Dr. Jose Costa to the VOMP Section as soon as a surgical pathologist can be found to replace him.

Dr. Timothy Triche joined the staff of the Ultrastructural Pathology Section in 1974 as a Senior Staff Fellow. He is a pathologist and electron microscopist and replaces Dr. Grimley who resigned in 1973.

Dr. Lucia Dunham and Dr. Katharine C. Snell retired in 1974. Dr. Maila Penttinen terminated as a Visiting Fellow on June 30, 1974.

Dr. Joy Burbach is a Medical Officer with Dr. Berard. Her appointment will terminate in June 1975. Dr. Risa B. Mann will start with Dr. Berard as a Staff Fellow in July 1975. Dr. Koji Namba from the University of Hiroshima started working with Dr. Berard as an International Research Fellow - Fogarty Center in July 1974. Dr. Shaw Watanabe will start as a Guest Worker with Dr. Berard in June 1975. Dr. Raul Braylan will continue in the Hematopathology Section as a Senior Staff Fellow during FY-76. He will be converted to a permanent staff position after he becomes a USA citizen later in 1975. During 1975, several other Guest Workers will work with the staff in the Laboratory of Pathology, pending approval of support funds.

The staff were either senior authors or co-authors of 41 papers and 13 abstracts published in 1974. Thirteen papers have been published through March of 1975 and there are currently 48 papers in press or submitted for publication.

Surgical Pathology Section:

Dr. Powell and Dr. Costa together with the residents and other pathologists are responsible for the diagnostic services in Surgical Pathology. Approximately 6563 specimens or biopsies were accessioned. Included are approximately 2760 bone marrows sectioned and processed in the Laboratory. Dr. Powell, Dr. Costa and the resident staff make rounds with the staff of the Surgery Branch. They project and discuss slides from most of the surgical biopsies and operative specimens at the weekly Surgical Pathology Conference. Other conferences based on surgical or biopsy material are conducted with clinical groups including the Hematology-Lymphoma conference and the Lymphoma staging conference (Drs. Berard, Jaffe, Braylan), the Radiation Therapy-Pathology conference (Dr. Costa), and the Pathology-Pediatric Oncology conference (Dr. Triche).

Postmortem Section:

Dr. Thomas, the pathology staff and the residents were responsible for the 157 autopsies performed on Clinical Center patients. This number represents approximately 95% of the total number of patients who died in the Clinical Center. The staff also reviewed submitted slides and protocols of approximately 49 former Clinical Center patients who died elsewhere. In addition, there was review and study of autopsy sections from more than a hundred other cases autopsied elsewhere but submitted as part of special studies. These special studies include material from the vinyl-chloride workers, from the national survey of angiosarcomas of the liver, and from referral of lymphomas to Dr. Berard and his staff and referral of cardiovascular cases to Dr. Roberts and his staff. A gross review of organs is held with the residents and clinical staff each morning at 8:30 a.m. and the staff and residents present selected autopsies at numerous clinico-pathologic conferences. Many specimens of a variety of human tissues, including the eyes which are routinely removed, are regularly furnished to NIH scientists in various laboratories.

Publications of Studies on Human Material:

Slightly more than half of the 1974 publications by the staff and residents were based on human tissues or some aspect of human disease. These include: mycosis fungoides (Thomas-28); Burkitt's tumor (Berard-38); other malignant lymphomas and leukemia (Berard-3,4; Thomas-12,16; Jaffe and Berard-14; Jaffe-21; Braylan-27; Braylan, Jaffe and Berard-30); osteogenic sarcoma (Lotz-7); pigmentary retinal degeneration (Rabson-9); extra-adrenal paraganglion tumors and hyperplasias (Grimley-11); isolation of Herpesvirus varicellae from human dorsal root ganglia (Bastian and Rabson-15); Sjogren's syndrome (Powell-19,41); leukemia and lower-motor-neuron disease (Costa, Rabson, Tralka-20); retinoblastoma (Rabson, Chu, Tralka-23); cytomegalovirus hepatitis (Henson, Grimley-1); isolation of a papovavirus from brain tumor and urine of a patient with Wiskott-Aldrich syndrome (Rabson-32); and fine needle aspiration of tumors (Chu-35). Reference numbers are to the list of 1974 publications.

Other Research Activities by Staff in Surgical Pathology and Postmortem Sections:

In addition to his diagnostic services in Surgical Pathology, Dr. Costa has continued with research in collaboration with Dr. Rabson in VOMP. Included are studies on the effect of corticosteroids on the growth of Herpesvirus type 2 in various cultures (enhanced production of virus in 3T3 cells and interference of viral release in Swiss mouse fibroblast cultures). He has collaborated with Dr. Herman and Ms. M. Cassidy to measure DNA content, with the Los Alamos Cell Sorter, of blood lymphocytes stimulated by PHA and PPD. Dr. Henson reviewed fatal cases of adenovirus pneumonia from the AFIP files and is studying NIH material with adenovirus infection. He has developed immunohistochemical methods to study various types of myosin in rhabdomyosarcomas and other types of sarcoma. Dr. Powell is studying the histologic alterations in squamous cell carcinoma produced by methotrexate and, with Dr. Allen Cheever, is completing a study of pulmonary diseases in an autopsy population in Cairo, Egypt. He is also collaborating with animal studies on testicular changes in zinc deficiency and sexual maturation in bulbectomized female rats. Ms. Tommie Sue Tralka, in collaboration with Dr. Rabson and Dr. O'Conor, has examined a number of human tissue cultures by EM. Dr. Thomas and Dr. H. Popper are continuing their studies of hepatic fibrosis and angiosarcomas which occurred in workers exposed to vinyl chloride and these studies are being extended to include mice exposed to various levels of vinyl chloride. The residents are studying a number of human diseases including: systemic mucormycosis and other bacterial and fungal infections (Dr. Kasten); dysgerminoma of the hypothalamus (Dr. Kolins); idiopathic cardiac failure and especially its possible relationship to viral myocarditis (Dr. Orlando); renal abnormalities due to myeloid metaplasia (Dr. Orlando); hyperlipoproteinemias in patients with coronary artery disease (Dr. Orlando); benign and second primary neoplasms in patients with breast cancer and in patients with malignant lymphoma (Dr. Tomaszewski).
Dr. Robert Stern has an especially active research program supported by his laboratory in NIDR. His projects include study of mechanism of action of phleomycin-bleomycin, development of an assay for ribonuclease activity, and mRNA dependent cell-free synthesis of collagen in a Krebs ascites culture system. He also is studying various types of collagen synthesized in a variety of human tumors and in various types of liver fibrosis produced by vinyl-chloride, CCl_4, Schistosomiasis, bleomycin and radiation.

Laboratory Services for Human Tissues:

Miss Bowling and her staff continue to provide excellent histological slides and special stains for human tissues. She also provides valuable consultation on special staining and preparation of human tissues to many Clinical

Associates and other NIH scientists. During the year Dr. Berard and Miss Bowling produced a 30 minute videotape on "Overcoming Technical Errors in the Histologic Preparation of Lymph Nodes" for the Professional Education Series of the American Society of Clinical Pathologists. This videotape will be distributed to many pathologists and histologic technologists throughout the USA.

Photography services for human tissues are provided by Mr. Edwards and Mr. Banks, who in addition, maintains the slide files which contain more than a million slides. Immediate access to these and to the gross and microscopic photographs is invaluable for patient diagnosis and follow-up and for an increasing number of clinico-pathologic studies.

Cytopathology Section:

Dr. Chu and the staff are responsible for all diagnostic reports on exfoliative cytology specimens which include sputum, urine, cervical-vaginal smears, CNS fluid, aspirated material, etc. Dr. Clyde Dawe is also participating in this diagnostic service. In addition, she and her staff are responsible for karyotyping and reporting on chromosome abnormalities. Approximately 5408 accessions (12,231 smears), including chromosome analysis specimens and smears, were processed and examined in 1974. This is approximately a 10% increase in work-load over the previous year. The evaluation of fine needle aspiration for cytologic interpretation is continuing in collaboration with the staff of the Surgery Branch. Dr. Chu and her staff have organized workshops on Aspiration Cytology for the American Society of Cytology meetings for the past 3 years. Other research projects include studying chromosome abnormalities in a variety of human tissues including cultured blood lymphocytes infected with cytomegalovirus.

Automation of Cytology:

Dr. Herman and his staff have the Los Alamos Cell Sorter and the Coulter volume equipment in full operation and have completed several projects during the past year. These include the determination of the Coulter volume distribution of cells from normal and abnormal gynecologic specimens, the analysis of two stains (Acridine orange and Mithramycin) which appear to distinguish normal from abnormal cytology specimens, and the use of flow analytic techniques to evaluate blastogenic response of lymphocytes to different antigens. Papers reporting these studies are now in press. Dr. Herman, Dr. Bunnag, Ms. Cassidy and Ms. Fowlkes have also developed extensive plans for scientific studies with these instruments for the coming year. All of these accomplishments are especially noteworthy because, during this year, Dr. Herman has continued residency training in the Clinical Pathology Department and, in addition, he and the staff have major extramural responsibilities for the contract program in the automation of cytology.

Ultrastructural Pathology Section:

Dr. Triche came to the Laboratory of Pathology in July 1974 and has spent this year reorganizing the Ultrastructural Pathology Section and developing specific plans for research and diagnostic studies with EM, SEM and freeze-etch electron microscopy. During the past 6 months 47 diagnostic specimens of human tissue were processed and EM studies of 24 of these were completed and reported. These included EM evaluation of hepatic fibrosis (with Dr. K. Nanba) from 5 vinyl chloride workers; the results of these studies are being reported at the AFCR meeting in May. Dr. Triche's plans for the use of the EM and SEM equipment include collaboration with several staff members in the Laboratory of Pathology and, in particular, with Drs. Braylan and Nanba in the Hematopathology Section. Dr. Triche also has developed a teaching program for residents in EM and SEM. It is anticipated that the freeze-etch equipment will soon be delivered and installed in the Ultrastructural Pathology Section, so that research with freeze-etch can be fully developed in conjunction with current EM and SEM projects. Dr. Triche is a skilled diagnostic pathologist and participates in all phases of the diagnostic services including review of surgicals and autopsies with the residents.

Dr. Henson's publications on human diseases were listed above. During the past year he published his studies on the ultrastructural localization of H. simplex virus antigens on rabbit corneal cells using sheep antihuman IgG-antihorse ferritin labelled hybrid antibodies. He is developing variously labelled hybrid antibodies to study embryonic antigens on cells and to study various types of myosin. He also participates in the diagnostic services and has major responsibilities for pathology data processing and the development of coded nomenclatures for the College of American Pathologists (SNOMed) and for the WHO (ICD-0).

Hematopathology Section:

Dr. Berard, Dr. Jaffe, Dr. Braylan and their co-workers, Dr. Nanba and Dr. Burbach, have major responsibilities for the diagnosis of patients with diseases of the hematopoietic and lymphoreticular tissues. It is estimated they have reviewed and been responsible for the diagnosis of between 500 and 600 cases of malignant lymphoma this past year. A minority of these are Clinical Center patients; the rest are derived from referral of cases from the ECOG, the gallium-67 study of cases by Oak Ridge Associated Universities, the American Burkitt Lymphoma Registry, and referral from pathologists in the U.S. and in foreign countries. In addition, Dr. Berard and his staff have developed a coordinated research program for the study of the morphology and the immunologic and functional characteristics of lymphoreticular cells in benign and malignant human diseases. The success of this broad-based research program is indicated by the relatively large number of scientific publications during 1974, 1975 and currently in press (see attachments). Included are studies on the light microscopy, immunologic surface receptors, SEM surface morphology and, in some cases EM morphology, of cells from patients with leukemic reticuloendotheliosis (hairy cell leukemia), Hodgkin's disease, T-cell lymphomas, childhood lymphoblastic lymphoma, nodular lymphocytic lymphomas, well-differentiated lymphocytic lymphomas, and a case of angiomatoid lymphoid hamartoma. As noted in last year's report, the results of these studies add new and important functional information to knowledge about the normal and neoplastic cells of the malignant lymphomas and leukemias. Dr. Berard and his staff have also participated in all phases of the diagnostic services in surgical pathology, autopsy pathology and cytopathology including the teaching of residents.

They have had an especially heavy schedule of lectures at Universities and at scientific meetings in the USA and in other countries. During the past year, Dr. Berard has also assumed supervisory responsibility for the Ultrastructural Pathology Section and has assisted Dr. Triche in the reorganization of that Section.

Comparative Oncology Section:

Drs. Dawe, Banfield and Stanton are the professional staff members of this Section. Dr. Lucia Dunham and Dr. Snell retired in 1974. Dr. Dawe's studies on polyoma virus-transformation of mouse salivary gland epithelium in cell and organ cultures have continued. He has shown that transformation of the epithelium can occur when the epithelium is separated from salivary mesenchyme by a millipore filter. Three continuous cell lines of such transformed cells have been established and, in transplantation experiments in mice, the tumors from the transformed cell lines are being compared to polyoma-induced tumors *in vivo*. Also experiments during the past year showed transformation in transfilter cultures with epithelium on both sides of the filter. This was an unexpected finding, because it represents the first transformation of epithelium in the absence of mesenchyme. Dr. Dawe, in collaboration with Dr. Banfield and Dr. Sonstegard, have recently discovered cytoplasmic structures by EM in the cells of a malignant lymphoma of the northern pike. These "cylindroid lamella-particle complexes" are similar to cytoplasmic structures found in the cells of hairy cell leukemia and in human embryonic cells transformed *in vitro* by EB virus from a case of Burkitt's lymphoma. Studies of these ctyoplasmic complexes in the pike lymphoma cells are continuing in order to find out how they are formed and to determine whether they are derived from DNA or RNA. Dr. Banfield, in collaboration with Dr. Sokoloff and Dr. Hough (NIAMDD), used the SEM as an electron probe for the energy dispersive x-ray analysis of the tide marks and osteochondral junctions of the articular regions of joints in humans and animals. In these experiments they demonstrated an excess of calcium but not of sulfur or phosphorus in the tide marks. In similar experiments they were able to show abnormal inclusions containing iron associated with phosphorus in the chondrocytes of articular cartilage in patients with advanced hemophilic arthropathy. Dr. Banfield has also continued his studies on the size of the dense granules present in various pox viruses. Dr. Stanton has continued with his long-range experiments on tobacco condensate carcinogenesis and fiber carcinogenesis. He has now shown that both mice and rats develop mesotheliomas in response to fibrous glass and asbestos. He has also shown that tremolite, silicon carbide and certain fibrous talcs are carcinogenic and that response seems related to fiber size. These experiments are significant because they indicate the potential carcinogenic hazards for man of exposure to many materials of small particulate size in his work and in his environment.

Mr. Ralph Isenberg provided photographic services for the experimental studies and photomicrography for publications by the staff of the Laboratory of Pathology.

Viral Oncology and Molecular Pathology Section:

Dr. Nathaniel Young joined the staff of VOMP to replace Dr. Friedman in 1974 and was appointed Head of the Section after Dr. Rabson became Director of DCBD. The EM laboratory in room 2N103 and two technologists, formerly supervised by Dr. O'Conor, were assigned to Dr. Young and VOMP. Dr. Rabson with Dr. Takemoto and their associates isolated a human papovavirus from a reticulum cell sarcoma of the brain and from the urine of a patient with Wiskott-Aldrich syndrome. This virus has been cultured, described by EM and its oncogenicity is being studied in animals. Dr. Rabson, with Dr. Char, has completed a series of experiments on cell-mediated immunity, using a ^{125}IUDR cytotoxicity assay system against a long-term tissue culture of retinoblastoma cells, in 20 patients with retinal degenerations and 14 patients with various stages of retinoblastoma. Most of the patients with retinal pigment degenerations showed increased cytotoxicity. These findings are consistent with animal experiments which show that parts of the retina are antigenic and suggest that cellular immune processes may play a role in the pathology of retinal degenerations. Increased lymphotoxicity was also observed in patients with retinoblastomas and this cell-mediated immunity may be directed against retinoblastoma tumor antigens. *Herpesvirus varicellae* was isolated from both the dorsal root ganglia and from cutaneous herpetic lesions in a patient with malignant lymphoma. Dr. Rabson with Dr. Levinson and associates studied the mechanism of inactivation of *H. simplex* types 1 and 2 exposed to thiosemicarbazones and found that some of these viruses are susceptible to exposure to heavy metals. He has continued, in collaboration with Dr. Lewis, to study the *in vivo* and *in vitro* carcinogenicity of non-hybrid adenovirus 2 and various non-defective Ad2-SV40 hybrids. The results indicate that transformation of hamster cells by Ad2-SV40 hybrids are caused by the Ad2 genome and not to the SV40 genome. With Dr. Albert and associates, Dr. Rabson has studied the morphologic, biochemical and cytogenetic characteristics of a cell culture of retinoblastoma cells which have been maintained in tissue culture for the past 3 years. No viral particles were seen in the cultured retinoblastoma cells, but activity of an RNA-directed DNA polymerase similar to that in oncogenic RNA tumor viruses was demonstrated. Other experiments included study of the production of human placental alkaline phosphatase from a culture of human tumor cells (with Dr. Rosen) and study of a new contrast material, Lipiodol U.F. with a surface-active agent, used for hepatosplenograms (with Dr. Vermess). Dr. Young's plans for experimental work includes study of the structure and assembly of vaccine polioviruses, EM of heteroduplex viral RNA, cell-mediated immunity to herpesvirus antigens (with Drs. Herman and Costa), immunochemical characteristics of polypeptides of papovaviruses and enteroviruses and, in collaboration with Dr. Rabson and Dr. Costa, continuing study of the biology of a human papovavirus isolated from a brain tumor. In prèliminary studies on the histopathology of Machupo virus, the etiologic agent of Bolivian hemorrhage fever, in *Calomys callosus*, Dr. Young has showed that the virus produces profound destruction of lymphoreticular tissues and severe hemolytic anemia.

Pathological Technology Section:

Miss Sanders and four of her staff moved to Frederick in 1974 and have established a histopathology laboratory for processing animal tissues at the

FCRC. This laboratory is now fully in operation and has established a training program for histology technicians for Litton-Bionetics employees. The other five staff members of Miss Sanders' laboratory remain in Bldg. 37. The two groups together produced nearly 30,000 slides for approximately 90 different NCI scientists during 1974. The Baker contract for processing microscopic slides and storage of paraffin blocks was not renewed because all blocks have not been stored at the FCRC and because of the successful operation of the histopathology laboratory at Frederick. Plans are being developed to provide EM sections and facilities for electron microscopy to NCI investigators at the FCRC histology laboratory.

Project No. Z01 CBD 00504-19 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An Electron Optical Investigation of Normal and Pathologic Tissue

Previous Serial Number: Same

Principal Investigator: William G. Banfield, M.D.

Other Investigators: G. Kasnic, C.J. Dawe, W. Morgan, C.W. Lee, M. Ohmori

Cooperating Units: Okayama University Medical School, Okayama, Japan

Man Years:

Total: 3/4
Professional: 1/2
Others: 1/4

Project Description:

Objectives: 1. To describe and interpret the ultrastructure of selected pathologic states and to compare the results with suitable controls.

2. To look for and study the inciting agent or accompanying agent in neoplastic and other disease states using human, animal and tissue culture.

Major Findings: 1. Further information on the dense granule of the pox viruses was obtained and some of the results reported at the 8th International Congress on Electron Microscopy in Canberra, Australia. The pox viruses studied could be divided into three statistically significant groups on the basis of the size of the dense granule present in the mature virus particle.

I.	Shope Fibroma	largest 227 $(nm)^2$
II.	Rabbit Myxoma Rabbit Pox Cowpox Canary Pox	
III.	Vaccinia	smallest 158 $(nm)^2$

It was also found that the dense granule was significantly smaller in the mature forms than in the developmental forms or than in the forms undergoing uncoating. Attempts at characterizing its chemical nature have not been successful. Quantimet measurements of area were compared with the same measurements of area using a measuring magnifier graduated in tenths of millimeters. The quantimet values were significantly smaller in most instances.

2. A cylindroid lamella-particle complex in malignant lymphoma of northern pike has been studied further in collaboration with Dr. Dawe. This structure is similar to those described in hairy cell leukemia.

Significance: (NCP Objective 5, Approach 4) 1. The dense granule may be of fundamental functional importance in the development of the virus and in its effects on the host.

2. A study of the cylindroid structure in the transmissible pike lymphoma when correlated with the studies on the similar structure found in human "hairy cell" leukemia may lead to a better understanding of the latter.

3. This will be the first publication of any atlas on the ultrastructure of the mosquito. See publications.

Proposed Course: A major effort will be made to characterize the granule and cylindroid lamella particle complex chemically by histochemical techniques and to assess the latter's possible relation to the structure in "hairy cell" leukemia.

Keyword Descriptors:

Electron microscopy, histochemistry, computers, computerized area measuring device, pox virus, tissue cultures, animals, viral oncology

Publications:

1. Ohmori, M., and Banfield, W.G.: The Ultrastructure of the Mosquito, Aedes aegypti (L). Tokyo, Saikon Publishing Co., Ltd., 1974, 169 pp.

2. Banfield, W.G., Kasnic, G., and Lee, C.W.: The dense granule of the pox viruses. 8th International Congress on Electron Microscopy, Canberra, Australia, August 25-31, 1974. Proceedings 2: 638-639, 1974.

Project No. Z01 CBD 00504b-20 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An Investigation of the Collagenous Connective Tissues and Elastin

Previous Serial Number: Same

Principal Investigator: William G. Banfield, M.D.

Other Investigators: C.K. Lee, L. Sokoloff, C.W. Lee, A. Hough

Cooperating Units: NIAMDD, Lab. Exp. Path., Section on Rheumatic Diseases; Dept. Path., Vanderbilt Univ., School of Med., Dept. Path., Health Sciences Center, State Univ. of New York, Stony Brook, N.Y.

Man Years:

Total: 1/2
Professional: 1/2
Others: 0

Project Description:

Objectives: To elucidate the normal and abnormal structure of elastin and collagen and their associated cellular and intercellular components.

Major Findings: 1. In collaboration with Dr. Hough and Dr. Sokoloff the scanning electron microscope was used as an electron probe for the energy dispersive x-ray analysis of the tide mark and osteochondral junction and adjacent cartilage and bone from the articular regions of joints; human and animal. The tide mark, in decalcified sections, had an excess of calcium but not of sulfur or phosphorus. The calcium excess may explain its hematoxylinophilia. The osteochondral junction does not seem to be hypercalcified as reported in the literature.

2. Also, in collaboration with Hough and Sokoloff we have done an EDAX (energy dispersive analysis by x-rays) study of cartilage in hemophilic arthropathy using the scanning electron microscope as an electron probe. Abnormal inclusions containing iron associated with phosphorus are present within the chondrocytes in advanced hemophilic arthropathy.

Significance: (NCP Objective 5, Approach 2,6) More important than the first data indicating the probable cause of the hematoxylinophilia of the tide mark and the first of two positive identifications of iron chondrocytes of hemophilic arthrophy is the demonstration of the potential of the scanning electron microscope when used as an electron probe to perform non-destructive elemental analysis on microscopic structures.

Proposed Course: 1. Apply the scanning electron microscope with the energy dispersive x-ray analyzer to the analysis of connective tissues, particularly elastin and elastotic degeneration (senile elastosis).

2. With Dr. Hough prepare an article "X-ray Analysis of Mammalian Joints" for a Hayet Monograph.

Keyword Descriptors:

Electron microscope, scanning electron microscope, energy dispersive analysis, cartilage, collagen, bone, joint, osteoarthritis

Publications:

1. Hough, A.J., Banfield, W.G., Mottram, F.C., and Sokoloff, L.: The osteochondral junction of mammalian joints - An ultrastructural and microanalytical study. Lab. Invest. 31: 685-695, 1974.

Project No. Z01 CBD 00505-05 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Evaluation of Potential Pulmonary Carcinogens, Asbestos, Tobacco, Silicates and Heavy Metals and their Relation to the Physiological Status of the Lung

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: E. Miller, M. May, and B. Sanders

Cooperating Units: Johns-Manville Research and Engineering Center, Manville, New Jersey; Owens-Corning Fiberglass Corp., Toledo, Ohio; Dr. Fred Bock, Roswell Park Memorial Center for Cancer Research; Pharmacopathics Inc., Contract NCI DCBD-N01-CB-33887

Man Years:

Total: 2/5
Professional: 2/5
Others: -

Project Description:

Objectives: 1. Investigations into pulmonary carcinogenesis with tobacco products and other potential pulmonary carcinogens for man.

2. Investigations into the factors affecting development of mesotheliomas experimentally induced with asbestos, fibrous glass and related fibrous minerals.

Major Findings: Analysis of a series of 17 experiments with 17 different size distributions of glass fibers indicates a close correlation between carcinogenesis and fibers with diameters less than 2.5 μm and lengths greater than 64 μm. Work is progressing to evaluate the impact of submicroscopic fibers on this response. Several other facts have become evident during the year which require final evaluation. These include: (1) Asbestos has been established as carcinogenic in the peritoneal and subcutaneous tissues of the rat. (2) Both asbestos and ultra fine fibrous glass have been established as carcinogens for the pleura of the mouse. (3) A variety of other fibers have proven to be carcinogenic on the pleura of the rat. These include tremolite, fibrous talc, potassium titanate fibers, sodium-aluminum carbonate fibers, fibrous gypsum and fibrous silicon carbide. All of these compounds have dimensional characteristics which fit into the concept recently developed concerning the relation of structure to carcinogenicity.

Examination of potential chemical pulmonary carcinogens is being carried out using the intrapulmonary pellet method. We hope to extend studies on tobacco smoke condensates to more specific fractions and to further examine other potential carcinogens such as extracted products of air-sample collected air-pollutants and specific chemicals such as benzene, carbon tetrachloride and the many specific carcinogenic hydrocarbons found in low levels in cigarette smoke and general atmospheric pollutants.

In the studies with intrapulmonary deposits of cigarette smoke condensates, a large series involving more than 4000 rats has been initiated to determine the carcinogenic potential of 9 highly defined fractions of cigarette smoke condensate isolated by the Agriculture Research Center, USDA, Athens, Georgia.

Significance: (NCP Objective 1, Approach 1)

Proposed Course: A large-scale contract, funded by DCCP, has been undertaken to test the screening ability of the pulmonary pellet method to detect differences between cigarette smoke condensate from cigarettes with high and low nicotine content. In our own laboratory, we intend to test the method with several innovative cigarette smoke condensates and to explore the system with smoke condensates of other types that are common air pollutants. Briefly our plans call for testing nicotine and various fractions of cigarette smoke condensate and the condensate of cigarette smoke from cigarettes with altered or reduced tar and nicotine content as well as extracts from specific air pollutants and other potential pulmonary carcinogens that may be lipid soluble.

Work with fibers involves the tedious assessment of fiber sizes and separation of fibers into specific size groups for testing utilizing asbestoses, glasses, aluminum oxide, silica, iron and carborundum fibers. The effects of reduction in durability as a result of pyrolysis and the effect on surface properties by fiber coating will be investigated as time and space permit. Electron microscopy of candidate specimens of fibers and fine structure analysis of the early metaplastic alterations in pulmonary epithelium are in progress with the aim of understanding the preneoplastic changes that may be related to the induction of cancer.

In regard to fiber carcinogenesis, asbestos causes cancer not because it is asbestos, but because it contains durable fibers of exceptionally small diameter that are of exceptionally long length. The important corollary to this fact is that any durable fiber of comparable dimension may be as carcinogenic as asbestos. Our current efforts are being directed to searching and testing many types of fibers that are liable to contact with humans in one way or another. In particular, aerosols from dust-producing materials ranging from fertilizers to pollens as well as large diametered insulation materials are being examined. Furthermore, fibrous materials ingested orally are being studied. In general, testing consists of intrapleural application as previously described. However, we have recently begun a series of studies on the effect of intravenously administered fibers. This relates directly to the problem of orally ingested fibrous particles since absorption into the blood stream has been demonstrated in the rat.

Keyword Descriptors:

Carcinogenesis, environmental carcinogens, fiber carcinogens, asbestos, fibrous glass, cigarette smoke condensate, tobacco, mesothelioma, carcinoma of the lung

Publications:

1. Hirano, T., Stanton, M., and Layard, M.: Measurement of epidermoid carcinoma development induced in the lungs of rats by 3-methyl-cholanthrene-containing beeswax pellets. J. Natl. Cancer Inst. 53: 1209-1219, 1974.

2. Stanton, M.F., Layard, M.W., and Miller, E.: Studies with fractionated cigarette smoke condensate in beeswax pellets implanted in the lungs of rats. In Karbe, E. and Park, J. (Eds.): Experimental Lung Cancer. New York. Springer-Verlag, 1974, pp. 392-400.

Project No. Z01 CBD 00516c-10 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Morphologic Studies on Neoplasms and Preneoplastic Conditions in Relation to Pathogenesis, Etiology and Diagnosis

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: E. Miller, M. May, and B. Sanders and Staff

Cooperating Units: Richard Adamson, M.D. and Staff (LCP)

Man Years:

Total: 2/5
Professional: 2/5
Others:

Project Description:

Objectives: A study and accessioning of cancer in laboratory animals. Collection and development of competence in special areas of cancer morphology. Studies of histopathological and fine structure alterations related to the pathogenesis of disease induced by oncogenic viruses and other carcinogens.

Major Findings: An ongoing program has been the development of special competence in the histopathological and fine structural aspects of tumors of the lung, bone and mammary glands of rodents. Collection and examination of induced and naturally occurring bone tumors has been in progress for more than 5 years and will continue.

The collection and development of competence in lung tumors is essential for the work previously described and additionally serves as an aid to other investigators in the area and to the NCI Registry of Experimental Cancers. A current project on which Ms. May serves is the study of the fine structure of the early squamous metaplasia preceding carcinoma at the site of implanted carcinogen pellets and the comparison of this change with the metaplasia associated with non-specific pulmonary inflammation which does not progress to cancer.

Interest in mammary neoplasia in rats is an offshoot of current long-term pulmonary experiments. Since over 50% of female strain OM rats develop mammary tumors in the second year of life it is necessary to surgically remove these in our experimental groups. As a result we have correlated data on incidence, growth rate and morphology in several hundred excised mammary neoplasms.

Significance: (NCP Objective 1, Approach 2)

Proposed Course: We have through the past 5-6 years acquired an extensive catalog of neoplasms of all types that develop in aged female Osborne-Mendel rats. This data will be collated in an effort to establish normal incidence rates for our colony. In addition, time will be devoted to training in electron microscopy, studies of neoplasia in lower animals and preparation of a monograph on lung tumours in rats.

Keyword Descriptors:

Animal models, mammary carcinoma, bone tumors, carcinoma of the lung, electron microscopy, preneoplastic lesions

Publications:

1. Stanton, M.F.: Tumors of bone. WHO Monograph Pathology of Tumours in Experimental Animals, vol. II, Tumours of the Mouse. In Press.

2. Stanton, M.F., and Stewart, H.L.: A review of pathology of tumours in laboratory animals. Volume 1: Tumours of the Rat, part 1. Environmental Research and by the Committee on Animal Models and Genetic Stock. In Press.

3. Stanton, M.F.: Tumors of the lung. WHO Monograph Pathology of Tumours in Experimental Animals, vol. I, pt. II, Tumours of the Rat. In Press.

Project No. Z01 CBD 00521a-05 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Investigations of Mechanisms of Tumor Induction by Polyoma Virus in Mice

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D., Ph.D.

Other Investigators: W.G. Banfield, W.D. Morgan, J.E. Williams, C. Lee

Cooperating Units:

Man Years:

Total: 6/10
Professional: 6/10
Others: -

Project Description:

Objectives: To identify the conditions necessary for the neoplastic response of mouse salivary gland epithelium to polyoma virus. Emphasis is on epithelio-mesenchymal interaction during tumorigenesis.

Previous work from this project has shown that:

1) The salivary gland tumors induced by polyoma virus are of epithelial origin. The strongest evidence for this was provided by experiments in which epithelium was consistently traced to be the source of the neoplastic cells by chromosome-tagging of the candidate cell types (Science 171: 394-397, 1971).

2) Salivary epithelium isolated from the mesenchymal cells of salivary gland failed to yield neoplasms when infected with polyoma virus and placed under conditions favorable to neoplastic development in vivo or in vitro (Int. J. Cancer 1: 419-450, 1966).

In view of these two major findings and ancillary evidence, a concept was developed to the effect that the polyoma-salivary tumor system is a 3-component system comprised of epithelium, mesenchyme, and virus, rather than simply of epithelium (responder) and virus (effector).

The current objective is to determine the mechanism by which the mesenchyme operates as an essential component in this system.

Methods Employed: To investigate the possible requirement of cell-cell contact between epithelium and mesenchyme for tumorigenesis, the response of epithelium to polyoma virus is studied in a trans-filter culture system in which epithelium and mesenchyme are separated from each other by a millipore membrane (0.45μ porosity, 20-25μ thickness) that is permeable to macromolecules but not to cells. This is done with a double-compartmented sealed tissue culture chamber devised by Mr. Morgan. Epithelium is placed on one side, and mesenchyme on the other side of a millipore filter that divides the chamber into 2 compartments, and both tissues are infected with virus. These trans-filter cultures are maintained for periods up to 1 year and more. At serial time intervals the millipore membranes are removed and results evaluated by 1) light and electron microscopy of the cells on the membranes; 2) observations of the behavior of the recovered cells in Leighton tube cultures; 3) observation of the tumorigenic capacities of the cells after subcutaneous transfer to newborn mice.

Major Findings: At last year's reporting, it was observed that among 22 polyoma virus-infected cultures of epithelium grown trans-filter from mesenchyme, morphological transformation occurred in the epithelium in 5 and in the mesenchyme in 1. Among 23 uninfected control cultures of the same type, no transformation was seen in either epithelium or mesenchyme.

Since then, we have obtained extremely interesting results in PV-infected cultures in which epithelium alone was grown on both sides of the millipore filters. In 3 of 19 such cultures, epithelial transformation occurred as judged by morphological and growth-in-vitro criteria. Two of these cultures are being continued after transfer from the filter chambers to Leighton tubes, in attempts to grow them as continuous cell lines. As soon as these transformed lines grow well enough to provide cells for animal inoculation, they will be tested for tumorigenicity in isogeneic mice.

Light microscopically, the epithelial cells transformed in the absence of salivary mesenchyme are not distinguishable from those transformed in the presence of salivary mesenchyme.

Since last year's report, we have succeeded in obtaining tumors in mice by transplanting epithelial cells transformed in transfilter cultures of epithelium opposite mesenchyme. The morphologic features of these tumors are somewhat variable; of much significance is the fact that the culture-derived tumors are not identical to salivary tumors induced in vivo. They tend to be more highly anaplastic, more invasive, and have less distinct epithelial features than do the tumors induced in vivo. The histological comparisons are not yet complete, however.

Significance: (NCP Objective 1, Approach 1,3) The finding that salivary epithelium, when grown on millipore filter, can be transformed by polyoma virus in the absence of mesenchyme promises to throw out our long-held concept that salivary epithelial transformation by PV requires interaction with mesenchyme. The old concept will definitely have to be discarded if the cultures of epithelium transformed in absence of mesenchyme prove to be tumorigenic. Assuming this will be the case, the observation is of much interest, because it suggests that the millipore filter as a substrate for salivary epithelium can in some way substitute for mesenchyme, the substrate on which epithelium normally rests. The present indications are that even though transformation of salivary epithelium can be affected by PV in the absence of mesenchyme, the resulting tumors will probably be different from those induced during epithelial-mesenchymal interaction. This is speculative, however.

Proposed Course: Present plans are to obtain repeated examples of transformation of salivary epithelium growing on millipore filter in the absence of mesenchyme, and to further characterize the tumors produced by epithelium transformed in this way. The influence of the millipore membrane itself will be given greater attention, as we already have some indication that the epithelium may occasionally transform in the absence of PV infection. This possibility must be examined critically, for if it is true, it suggests that the viral infection serves mainly to accelerate a process that occurs under certain culture conditions anyway.

In June or July of 1975, Dr. Kurokawa will probably join us as a Visiting Scientist supported by a Fogarty Fellowship. He will help to extend our studies of epithelial-mesenchymal interactions during epithelial carcinogenesis by starting an investigation of chemical carcinogenesis in skin and esophagus in intact organ cultures and in cultures of epithelium of these organs isolated from their proper mesenchymes. Dr. Kurokawa's Fellowship has been approved, but it remains to be determined whether it can be funded.

Keyword Descriptors:

Oncogenic virus (polyoma), transformation in vitro, organ culture, viral carcinogenesis, salivary gland tumors of mice, trans-filter epitheliomesenchymal interaction

Project No. Z01 CBD 00538-10 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Study of Phylogenetic Aspects of Neoplasia

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D., Ph.D.

Other Investigators: J.C. Harshbarger, W.G. Banfield, D. Small, and others

Cooperating Units:

Man Years:

Total: 5/10
Professional: 5/10
Others: -

Project Description:

Objectives: The continuing major objective of this project is to perform comparative studies of neoplasia in invertebrate and lower vertebrate animals. Such studies serve two main needs: 1) To gain a comprehensive view of the biology of neoplasia throughout the animal phyla and thus to achieve a more detached perspective of the problem of cancer in man; 2) To find and make use of neoplastic diseases in lower animals in 2 ways: a) by adapting them to experimental uses in studies of the properties and mechanisms of genesis of cancer; b) by using them as indicators of environmental tumorigenic agents possibly related to the epidemiology of human neoplasia.

Methods Employed: Neoplasms are collected, either in the field or through submittal from outside sources, and are studied morphologically and biologically. Light and electron microscopy are used with fixed material. Tissue culture, transplantation, and induction methods are applied to study phenomena such as regeneration, dedifferentiation, autotomy, graft rejection, tumor progression, and others. Much of this work is done in conjunction with the Registry of Tumors in Lower Animals at the Smithsonian Institution, for which the PI serves as Project Officer.

Major Findings and Activities: A. Much of the reporting investigator's work in this project has been concerned with organizational, advisory, and educational activities. Among these were:

1. Organization and chairmanship of a symposium on Environmental

Carcinogens in Feral Aquatic Animals at the XIth International Cancer Congress in Florence, October 20-26, 1974. The proposed program has been approved by the Program Committee of the Congress.

2. Organization and chairmanship of a symposium on Aquatic Animals as Indicators of Environmental Carcinogens, sponsored by the International Union Against Cancer. This meeting was held at University College, Cork, Ireland, October 16 to 18, 1974. The papers presented are being edited for publication in Vol. 21 of Progress in Experimental Tumor Research. The principal investigator is one of the guest editors for this publication.

3. Presented a paper entitled "Phylogeny, Ontogeny, and Oncogeny" at the Argonne National Laboratories symposium on Oncology, November 1974.

4. Since January 1974, have been serving as consultant to Board of Directors, The Upper Bay Survey. This survey is an ecological study supported by the State of Maryland's Department of Natural Resources for the purpose of identifying the impact of chlorinated hydrocarbon contamination on the ecosystems of the Chesapeake Bay. The survey will be concluded in April 1975.

B. Investigative Work.

1. The study of a chondrosarcoma in a corn snake, reported in 1973, has not been actively continued this year. The cell culture line derived from the chondrosarcoma is being maintained for possible future use. The observations on the properties of the cell line in culture will be included with a publication to be prepared by Dr. David Small of the Laboratory of Microbiology and Immunology, NIDR.

2. A culture cell line from a nephroblastoma in a rainbow trout has been isolated and maintained for 1 year. The line grows slowly at room temperature and can be subcultured at intervals of 1-2 months. The original spontaneous tumor, like several others we have seen in rainbow trout, was well-differentiated, showing only slightly atypical glomerulus and tubule formation. In culture, cells show epithelial as well as mesenchymal characteristics. During the past year, this cell line was placed in contact co-culture with dorsal spinal cord from 11-day mouse embryos, to see whether the nephroblastoma cells might be capable of tubulogenesis under this stimulation. (Normal metanephrogenic mesenchyme undergoes tubulogenesis in contact with dorsal spinal cord). There was no response of any type on the part of the nephroblastoma cells. Only a single set of cultures was studied, but excellent contact was established between spinal cord and masses of the cultured cells, and the result was unequivocally negative.

The only remaining experiment planned for this cell line is to transplant it intraocularly in adult rainbow trout. We may also try to transplant it to the kidney of young adult trout.

3. Search for further examples of cholangiomas in white suckers (Catastomus commersoni) continues. Another sampling from Deep Creek Lake is in progress as this report is being prepared. Since this tumor has been seen in only one fish since the discovery of 3 in 12 fish examined in 1963, it appears to be a rare neoplasm and may not be useful as an indicator of an environmental carcinogen. A total of more than 3,000 fish from many parts of the U.S. and Canada has now been examined in this project.

4. A preliminary histopathologic examination of gonadal tumors in carp has been initiated upon request from Dr. R. Sonstegard, of the University of Guelph, Canada. These tumors have been found at high prevalence rates in carp in certain locations, and are suspected to be associated with industrial pollution.

Significance: (NCP Objective 1, Approach 1,4) The field studies concerned with tumors in carp and white suckers are relevant to cancer prevention, in that they may lead to disclosure of environmental carcinogens. The fact that these species are both bottom-feeders suggests that presumptive carcinogens may be adsorbed to bottom sediments or concentrated in molluscs in the food chain of these fish. Heavy metals and chlorinated hydrocarbon pesticides are known to be carcinogenic, and are also found adsorbed to suspended as well as sedimented particulates in aquatic environments.

Proposed Course: The field search for cholangiomas in suckers will be continued as in past years. The results of the past 10 years' work in this project will be published (see publications).

The collaborative histopathologic study of gonadal tumors in carp will be extended until a satisfactory histogenetic classification can be accomplished. Further participation in this investigation will depend upon the outcome of this initial work as well as on Dr. Sonstegard's future field studies of these tumors.

The study of the trout nephroblastoma will be extended to include attempts to transplant the cell line to rainbow trout hosts, intrarenally and intra-ocularly.

Keyword Descriptors:

Neoplasms in lower animals, nephroblastoma in trout, cholangiomas in white suckers, gonadal tumors in carp, fish tumors

Publications:

1. Dawe, C.J., Sonstegard, R., Stanton, M.F., Woronecki, D.E., and Reppert, R.T.: Intrahepatic biliary duct neoplasms in Catastomus commersoni. In Homburger, F. (ed.): Tumors in Aquatic Animals, vol. 21 in the series Progress in Experimental Tumor Research. In Press.

2. Dawe, C.J., and Harshbarger, J.C.: Neoplasms in feral fishes: Their significance to cancer research. Symposium on Fish Pathology, Armed Forces Institute of Pathology, Washington, D.C., August, 1972. In Press.

Project No. Z01 CBD 00539-01 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Lysis of Chicken Red Cells by $P388D_1$ Mouse Tumor Cells

Previous Serial Number: None

Principal Investigator: Hillel S. Koren, Ph.D.

Other Investigators: W.D. Morgan

Cooperating Units: Immunology Branch, NCI, DCBD

Man Years:

Total: 2
Professional: 1
Others: 1

Project Description:

In this project antibody dependent cellular cytotoxicity is studied by time-lapse microcinematography and motion analysis. $P388D_1$ which is a mouse leukemia cell line with macrophage-like characteristics and chicken red blood cells that have been sensitized with trinitrophenyl, are the cell types used in the project. This study focuses on cytotoxicity and phagocytosis, either or both of which may occur when $P388D_1$ and sensitized chicken red cells come in contact with each other. Cells used in the study are prepared in the Immunology Branch and supplied by Dr. Koren; time-lapse microcinematography and motion analysis are carried out in this laboratory.

Objectives: The object of this project is to produce a visual record and analysis of the "killing" of chicken red blood cells (targets) by $P388D_1$ mouse leukemia cells (effectors).

Methods Employed: Cell suspensions of 5.5×10^5 $P388D_1$ cells which are grown in spinner cultures by Dr. Koren, are placed on glass in modified multipurpose tissue culture chambers. These $P388D_1$ cells are then cultured for 24 hours in the chambers to allow the cells to become well attached to the glass. Then all medium (Eagle's minimal essential medium with Earle's balanced salt solution, MEM) is removed from the chamber and replaced with MEM containing 7.5×10^5 sensitized chicken red cells. Photography begins as soon as all red cells have been perfused into the chamber. The red cells tend to linger in suspension before gravitating toward the upper surface of the attached $P388D_1$ cells. This lingering (some take 30 minutes to settle down) permits photographic records to be made of several target-effector contacts. Photographs are taken at 3 second intervals for 3 hour periods during several days, as multiple challenges of target cells are introduced into a single culture of effector cells. A split frame technique of photography is used which permits the recording of test and control cultures on the same 16 mm film. This method aids photographic analysis. Film is analyzed on a Vanguard Motion Analyzer.

Major Findings: Preliminary results of microcinematographic studies of $P388D_1$-chicken red cell antibody dependent cellular cytotoxicity showed that: 1) killing of red cells occurred in 0.5 to 2.0 minutes after contact was established with $P388D_1$ cells; 2) a single $P388D_1$ cell could kill several red cells; 3) approximately 60% of killed red cells in contact with $P388D_1$ were phagocytized in 2 to 12 minutes after firm contact was established; 4) $P388D_1$ cells were not killed by ingested chicken red cells, and 5) $P388D_1$ cells killed chicken red cells in the absence of phagocytosis.

Significance: (NCP Objective 6, Approach 1) Preliminary findings which show that killing and phagocytosis of chicken red cells by $P388D_1$ cells occurred within 2-15 minutes suggest that prolonged application of effector cells may not be necessary in antibody dependent cellular cytotoxicity systems, and that better understanding of time factors in this cytotoxicity system may suggest new models for research in cellular immunity.

Proposed Course: After further analysis of film on hand and time-lapse microcinematography of "killing" of chicken red cells by $P388D_1$ cells treated with iodoacetate, which prevents phagocytosis, data will be reviewed, after which Dr. Koren and Mr. Morgan will write a report.

Keyword Descriptors:

Visualization of killing and phagocytosis in antibody dependent cell-mediated cytotoxicity

Project No. Z01 CBD 00560-09 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electron Probe and Scanning Electron Microscope

Previous Serial Number: Same

Principal Investigators: William G. Banfield, M.D., Stephen Doty, Ph.D.

Other Investigators: K. Heinrich, and staff of the National Bureau of Standards, C.W. Lee, H. Tipton, R.C. Braylan

Cooperating Units: National Bureau of Standards and Biomedical Engineering and Instrumentation Branch, Division of Research Services

Man Years:

Total: 1/2
Professional: 1/2
Others: 0

Project Description:

Objectives: Application of electron probe and ion probe to the analysis of biological specimens.

Major Findings: A stage for direct observation of tissue sections and cathode luminescence in the scanning electron microscope has been built and is undergoing final test.

Significance: (NCP Objective 5A, 5B, Approach 2,3,6) 1. Publication No. 1. The preparation of ultrathin frozen sections allows for localizing the electron beam in the electron probe using transmission scanning. It also allows for immunological procedures and enzymatic reactions that cannot be done on fixed tissue. In addition, the ability to prepare frozen sections for conventional transmission electron microscopy opens the way for assessing artefacts introduced by fixation, dehydration and embedding.

2. The light microscope for the scanning electron microscope will allow for light optical microscopic visual centering of the object of interest under the electron beam and direct visualization of the cathode luminescence.

Proposed Course: 1. In collaboration with the National Bureau of Standards, continue to work out quantitative analytical methods for biology using the electron probe.

2. Apply the scanning electron microscope, with energy dispersive x-ray analytical attachment (EDAX), to the analysis of connective tissues, particularly elastin, and to the analysis of stratified squamous epithelium, squamous carcinomas and basal cell tumors with especial emphasis on sulfur.

3. With Dr. Braylan, study phagocytic activity of mononuclear cell preparations using EDAX to detect the element phagocytized. Correlation of this activity with surface morphology.

4. Use the light microscope stage constructed for the scanning electron microscope in a study of the cathode luminescence of elastic fibers.

5. Construct a light microscope stage for the ETEC scanning electron microscope to be used for energy dispersive x-ray analysis.

6. Apply EDAX analysis to collaborative efforts as they arise.

Keyword Descriptors:

Scanning electron microscope, energy dispersive analysis, cathode luminescence

Publications:

1. Doty, S.B., Lee, C.W., and Banfield, W.G.: A method for obtaining ultrathin frozen sections from fresh or glutaraldehyde fixed tissue. Histochemical Journal 6: 383-393, 1974.

Project No. Z01 CBD 00561-05 LP
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Collaborative Research and Other Activities

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: None

Cooperating Units: DCCP, FDA, IARC-WHO

Man Years:

Total: 1/5
Professional: 1/5
Others:

Project Description: (Other Activities)

Twenty percent of the normal work week is spent in the following activities: 1. Member, Smoking and Health Review Board-OD; 2. Member, Asbestos Protocol Subcommittee of the HEW Committee on Coordinated Toxicological Research and Related Programs; 3. Project Officer, NCI, DCBD-N01-CB-33887, Small Animal Holding Facility; 4. Project Officer, NCI, DCBD-N01-CB-43912, Hematoxylin Substitute Development; 5. Co-Project Officer, NCI, DCBD-N01-CB-33874, Registry, Tumors in Lower Animals; 6. Co-Project Officer, NCI, DCCP-N01-CP-3374, Standardized Lung Pellet Technique; 7. Member, Editorial Board Monograph Series, IARC-WHO, Lyon, France.

Proposed Course: The following continuing activities are anticipated. I am directly involved as project officer to a major contract with Battelle Northwest to examine response to intrapulmonary deposits of high and low nicotine cigarette smoke condensates. This contract is supported by the Smoking and Health Program which currently is in the office of the Director, NCI. I also act as a member of this program's Smoking and Health Review Board on other segments of the program. I also act as project officer on the contract with Louisiana State University to develop substitutes for hematoxylin and the contract with Pharmacopathics, Inc. on the long-term animal holding facility. Finally, I represent the NCI on an Interagency Working Group, headed by FDA, whose mission is to plan and follow through on a program to experimentally investigate the effects of orally ingested asbestos.

Project No. Z01 CBD 00852-22 LP
1. Laboratory of Pathology
2. Cytopathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Exfoliative Cytology Applied to Human Diagnostic Problems and Research Problems

Previous Serial Number: Same

Principal Investigator: Elizabeth W. Chu, M.D.

Other Investigators:

Cooperating Units:

Man Years:

Total: 6
Professional: 2
Others: 4

Project Description:

Objectives: The primary responsibility of the Cytopathology Section is to provide the staff of the Clinical Center with an accurate and complete diagnostic service in exfoliative cytology, diagnostic cytogenetics and also fine needle aspiration cytology. In addition, the Cytopathology Section collaborates in various clinical research projects evaluating therapeutic effects, hormonal status of patients when requested and development of better diagnostic techniques.

The specific objectives include:

1. Work to improve cytologic techniques in establishing diagnosis.
2. Expansion and improvement in fine needle aspiration cytologic technique.
3. To study and develop cytologic characteristics in aspiration cytology.

Methods Employed: 1. Special procedures involving Millipore filtration and special stains in addition to the standard Papanicolaou stain are required in many instances in our cytological materials.

2. Short-term cultures of peripheral blood, bone marrow cells and tumor cells found in body fluids are our standard diagnostic cytogenetic methods. Special techniques involving trypsin digestion with resultant G-Bandings (Giemsa bands) are also applied.

Patient Material: The figures compiled below are for the last twelve months.

March 1974 - February 1975

Type of Material Examined:	Accessions	Smears
Vaginal and Cervical	1,361	2,362
Other (Ascitic, pleural, gastric, prostatic, urine, bronchial washings, sputa, spinal fluids, sex chromatin, wound washings, breast, oral, aspirations, chromosome analysis, chromosome tissue analysis, and special procedures)	4,060	9,306
Total	5,421	11,668

Institutes	Accessions	Smears
NCI	3,775	8,119
NHLI	282	679
NIAID	228	671
NIAMDD	478	679
NIMH	60	126
NIDR	61	129
NICHD	300	731
NINDS	194	410
NEI	7	36
REF.	28	72
EHS(CC)	8	16
Total	5,421	11,668

Major Findings: Major contributions of the Cytopathology Section:

1. Provides prompt services and early diagnosis on cytological materials that are often undiagnosed.

2. Provides cytologic evaluation in the continual management of meningeal leukemia.

3. Urinary cytologic evaluation of therapeutic effects in the care of patients on long-term chemotherapy.

4. Providing chromosomal analysis for clinical studies.

5. Providing cytologic methods to research projects.

Significance: (NCP Objective 1, Approach 1,2,3) The diagnostic value of exfoliative cytology in the clinical management of various disease states (both infectious and neoplastic) is an accepted fact. In addition, aspiration cytologic techniques are useful in establishing diagnosis in selected cases thereby eliminating the more involved incisional biopsy.

Proposed Course: 1. Continue to provide a complete cytopathologic service for the entire staff of the Clinical Center in various research projects.

2. Development and improvement of new and established techniques within the laboratory is the goal.

Keyword Descriptors:

Cytodiagnosis, cytogenetics, tissue culture, accessions, smears

Publications:

1. Schour, L., and Chu, E.W.: Fine needle aspiration in the management of patients with neoplastic disease. Acta Cytol. 18: 472-476, 1974.

2. Wood, T.A., Jr., DeWitt, S.H., Chu, E.W., Rabson, A.S., and Graykowski, E.A.: Anitschkow nuclear changes observed in oral smears. Acta Cytol. In Press.

Project No. Z01 CBD 00854-19 LP
1. Laboratory of Pathology
2. Cytopathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Experimental Studies on Endocrine Factors, Cytogenetic Characters, and Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Elizabeth W. Chu, M.D.

Other Investigators:

Cooperating Units:

Man Years:

Total: 2
Professional: 1
Others: 1

Project Description:

Objectives: 1. Effects of Enovid in rodents.

2. *In vitro* viral effects of lymphocytes and fibroblasts.

3. Polyploidization of cells by Cytocalacin and its possible relationship in the induction of malignancies.

4. The value of aspiration cytology in the clinical management of thyroid gland diseases. (In collaboration with Dr. Jacob Robbins, NIAMDD - Clinical Endocrinology Branch)

Methods Employed: 1. Mice are fed with Enovid pellets. At end of one year's treatment the cervix that shows epidermization is minced and transplanted to young adult mice.

2. *In vitro* cells, lymphocytes and WI-38 cell line were infected with virus (herpes and cytomegalovirus) for different periods of time. The cells were then harvested for chromosomal analysis.

3. a) Cytocalacin was used to induce polyploid cells; the cells were then harvested at intervals.

b) Cytocalacin solution is painted on hamster cervix 5 days/wk. Vaginal smears are taken at monthly intervals for cytological examination.

4. Patients with cold thyroid nodules measuring more than 1 cm. needle aspiration will be attempted prior to institution of prednisone or thyroid suppression.

Major Findings: 1. All mice that were fed with Enovid pellets developed vaginal, cervical lesions consistent with severe epidermization suggestive of cancer. Experiments were repeated to reproduce the lesion for transplantation into two months old mice. At the present time, approximately two months after transplantation, no definite growths have been observed yet.

2. Chromosomal damages on established fibroblasts by herpes and sendai viruses have been reported by several authors. Our pilot studies on cytomegalovirus demonstrated severe pulverization effects on chromosomes. The characteristics and extent of the damaging effect is presently under study.

3. a) Cytocalacin A when added to established cell lines induced bi- and multi-nucleation. However, we have not been able to maintain the multi-nucleated cells beyond four months. The reason for this failure is uncertain.

b) Vaginal smears of hamsters reveal cytological atypia after 4 months' paintings of the cervices with Cytocalacin A. No definite malignant changes have been seen yet.

4. In the past year, 25 patients with cold thyroid nodules had aspiration cytology done. The aspiration diagnosis of these nodules ranged from positive readings of cancer (two patients), to Hashimoto's disease. Four of these patients had surgical removal of the nodules. The histological findings confirmed the cytological interpretations which included the two cancer patients.

Significance: (NCP Objective 1, Approach 1,2,3) 1. The presence or absence of deleterious effects of contraceptive pills is of paramount importance since millions of women are using it.

2. Viral damages to human cells carry significant implications. Chromosomal studies will prove the relationship between virus and cancer.

3. Presumably polyploidy is precursory to ultimate malignant state.

4. The aspiration cytologic techniques in selected cases afford a simple and inexpensive diagnostic procedure.

Proposed Course: 1. Continue to study chromosomal characteristics of viral infected cells.

2. To study *in vitro* different viral effects on established human cell lines.

3. Continue to study polyploidy of cells and its relationship to cancer.

Keyword Descriptors:

Animal inoculations, tissue culture, virus, exfoliative cytology methods

Publications:

1. Reid, T.W., Albert, D.M., Rabson, A.S., Russell, P., Craft, J., Chu, E.W., Tralka, T.S., and Wilcox, J.L.: Characteristics of an established cell line of retinoblastoma. J. Natl. Cancer Inst. 53: 347-360, 1974.

Project No. Z01 CBD 00517-34 LP
1. Laboratory of Pathology
2. Pathological Technology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pathological Technology Section

Previous Serial Number: Same

Principal Investigator: Betty J. Sanders

Other Investigators: Dr. L.B. Thomas and Dr. C.J. Dawe

Cooperating Units: FCRC-NCI - Project 14

Man Years:

Total: 10
Professional: 1
Others: 9

Project Description:

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

July 1, 1974 - June 30, 1975

Number of investigators	87
Number of pieces of tissue	26,829
Number of bottles of tissue	5,009
Number of blocks cut	15,758
Number of blocks cut serially	252
Number of frozen blocks cut	164
Number of blocks recut	676
Number of slides stained H & E	20,558
Number of slides stained special	4,593
Number of unstained slides	4,836

Contract Work - Baker Histology Laboratory (contract expired 6/15/74)

Number of slides stained H & E	7,705

Keyword Descriptors:

Routine stains, special stains, frozen sectioning, plastic sectioning

Honors and Awards:

Betty J. Sanders - Outstanding Performance Award - December 1974

Publications:

1. Sanders, B.J.: Animal histology procedures. In CRC Handbook of Laboratory Animal Science. Cleveland, Ohio, CRC Press, 1974, vol. II, pp. 119-193.

Project No. Z01 CBD 00853-22 LP
1. Laboratory of Pathology
2. Surgical Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Surgical Pathology and Postmortem Sections, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all Institutes in those investigations which involve the use and study of human pathological material. The staff of the Surgical Pathology and Postmortem Sections also carries on independent and collaborative research.

Staff:

Dr. Ralph D. Powell, Jr., Head, Surgical Pathology Section
Dr. Louis B. Thomas, Chief, Laboratory of Pathology and Head, Postmortem Section
Dr. Jose Costa, Staff Pathologist
Dr. Costan W. Berard, Head, Hematopathology Section
Dr. Raul C. Braylan, Staff Pathologist
Dr. Clyde Dawe, Staff Pathologist
Dr. Donald E. Henson, Staff Pathologist
Dr. Elaine S. Jaffe, Staff Pathologist
Dr. Timothy Triche, Staff Pathologist
Dr. Nathaniel Young, Staff Pathologist
+Dr. Richard W. Balasa, Resident
+Dr. Bernard L. Kasten, Resident
+Dr. Jerry Kolins, Resident
+Dr. Michael M. Orlando, Resident
+Dr. Robert Stern, Resident
+Dr. Maria M. Tomaszewski, Resident
*Dr. Allen W. Cheever, National Institute of Allergy and Infectious Diseases
*Dr. Robert M. Friedman, National Institute of Arthritis, Metabolism, and Digestive Diseases
*Dr. William C. Roberts, Chief, Laboratory of Pathology, National Heart and Lung Institute
*Dr. Marius Valsamis, Associate Professor of Pathology, New York Medical College, Center for Aging and Chronic Diseases

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+ - These physicians are full time Residents in the Laboratory of Pathology.

* - These Associate Pathologists spend part time in the activities of the Surgical Pathology and Postmortem Sections.

Project Description:

Objectives: The objectives of the Surgical Pathology and Postmortem Sections are: (1) to provide diagnostic services in pathologic anatomy; (2) to collaborate with National Institutes of Health investigators in research involving use and study of human material; (3) to conduct a residency training program in pathologic anatomy, and (4) to carry out independent research.

1. The service functions of the Sections during the period April 1974 through March 1975 included:

(a) 360 autopsy examinations. Included in this report is a table which shows the number of autopsies performed for each Institute. The residents perform nearly all of the postmortem dissections under supervision of the staff. A gross review is held by the staff and residents each morning at 8:30. The residents review the microscopic slides of each autopsy with one of the staff before completing the autopsy protocol.

The staff assists the residents in preparing for the numerous clinical conferences in which the Sections participate.

(b) 6643 specimens were accessioned in the Surgical Pathology Section. A complete listing of these specimens, by Institute, is given in the accompanying table. These specimens are initially examined and described by a resident and their reports are checked by the staff. Associate pathologists from other laboratories or sections are frequently consulted about problems in diagnosis in surgical pathology and/or assume responsibility for handling certain tissue removed for specific research purposes.

(c) Histological preparation and special procedures:

These functions are carried out under the direction of Miss Mary C. Bowling and a complete listing of specimens processed by Institutes is given in the accompanying table.

(d) Photographic Services of the Department:

A photographic record is made of the large amount of the pathological material which is handled and studied in the department. The photography is done by the staff and residents with the technical assistance of Mr. L. Kenzie Edwards who also maintains the equipment and carries out much of the photographic processing. Gross photographs and photomicrographs of the pathologic material are provided to the clinical staff on request, are used extensively for conferences and seminars and many are prepared for publication.

Project No. Z01 CBD 00853-22 LP

REPORT OF POSTMORTEM PATHOLOGY SECTION

April 1, 1974 through March 31, 1975

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMDD	NIAID	NINDS	NIMH	NIDR	NEI	NICHD	REF.	TOTAL
Autopsies performed	118	21	8	3	7	--	--	--	--	--	157
Autopsies submitted	29	16	3	1	--	--	--	--	--	154	203
Total autopsies	147	37	11	4	7	--	--	--	--	154	360
Blocks of tissue cut	9986	1129	909	350	661	--	--	--	15	532	13582
Blocks of tissue recut	554	41	25	41	145	--	--	--	--	7	813
Blocks serialled	--	--	--	--	--	--	--	--	--	--	--
Total blocks cut	10540	1170	934	391	806	--	--	--	15	539	14395
Slides made	12604	1330	1449	502	1028	--	--	--	15	3180	20108
Slides H&E stained	10706	1137	953	385	781	--	--	--	15	2304	16281
Slides special stained	1140	81	70	101	91	--	--	--	--	626	2109
Total slides stained	11846	1218	1023	486	872	--	--	--	15	2930	18390
Frozen sections	20	--	1	--	--	--	--	--	--	--	21

Project No. Z01 CBD 00853-22 LP

April 1, 1974 through March 31, 1975

REPORT OF SURGICAL PATHOLOGY SECTION

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMDD	NIAID	NINDS	NIMH	NIDR	NEI	NICH	REF.	RESEARCH	TOTAL
Specimens accessioned	4137	348	207	250	105	10	411	6	27	822	322	6643
Blocks of tissue cut	8863	580	642	453	296	12	282	17	71	488	13	11717
Blocks of tissue recut	671	40	56	55	52	1	12	1	2	28	8	926
Total blocks cut	9534	620	698	508	348	13	294	18	73	516	21	12643
Slides made	28791	1649	2404	1852	906	27	696	38	268	4381	59	41071
Slides stained H&E	18204	903	1388	1007	470	15	346	18	128	3777	17	26273
Slides stained special	4645	270	313	447	157	3	20	6	92	591	25	6569
Total slides stained	22849	1173	1701	1454	627	18	386	24	220	4368	42	32842
Frozen sections	340	37	120	13	25	--	--	--	9	10	--	554

TOTAL SURGICAL AND POSTMORTEM PATHOLOGY SECTION REPORT:

Number of blocks cut ----------------27038
Number of slides made ---------------61179
Number of slides stained H&E ---------42554
Number of slides stained special ---- 8678

Listed below is a breakdown of the work performed by Mr. Edwards from April 1974 through March 1975:

Time taking photographs: 535 hours, 40 minutes
Time processing: 283 hours

No. of black and white negatives - 4 x 5 inches: 1954

Color transparencies - 4 x 5 inches - gross: 2486
35 mm. transparencies - gross: 8572
Total 11058

Prints contract out - 4 x 5 inches: 8890

A large proportion of the photomicrography is done under the direction of Mr. Ralph Isenburg who provides professional assistance and facilities for the entire staff of the Laboratory of Pathology.

2. The collaborative activities of the staff and residents in the Surgical Pathology and Postmortem Sections are reflected in the summary of research activities listed below:

(a) Distribution of human organs and tissues:

At present there are approximately 50 scientists in the various laboratories at the National Institutes of Health who have requested particular tissues from surgical and postmortem services. On many occasions it is possible to furnish these investigators with fresh human material. A list of these investigators is kept by the secretarial staff and telephone calls are made to the various investigators whenever surgical or postmortem tissues are available.

(b) Conferences:

The staff and residents taken an active part in numerous Clinical Center and Institute conferences. In addition, there are several conferences regularly organized solely or jointly by the department, including a Surgical Pathology Conference, Joint Pathologists Conference, Neuropathology Conference, Lymphoma Staging Conference, Medical Breast Cancer Service Pathology Conference, Medicine Branch Slide Review Conference, Pediatric Oncology Conference, Radiation Therapy Conference, Gastrointestinal Disease Conference, Brain Cutting Conference and Gross Autopsy Conference.

A. Dr. Ralph D. Powell, Jr.:

1. The pathology of trophoblastic disease, a chapter in the monograph "Choriocarcinoma and Related Trophoblastic Disease" being prepared for publication by Dr. R. Hertz. In collaboration with Dr. L.B. Thomas, Laboratory of Pathology, National Cancer Institute.

2. A study of the natural history of bronchogenic carcinoma and the effect of chemotherapy and radiotherapy on survival with special emphasis on oat-cell carcinoma. In collaboration with Drs. R.E. Johnson and K.B. Lamoureux, Radiation Branch, National Cancer Institute.

3. Histologic alterations in squamous carcinoma of the head and neck region after methotrexate chemotherapy. In collaboration with Drs. P.B. Chretien, Surgery Branch, National Cancer Institute and A.S. Ketcham, University of Miami, School of Medicine, formerly Surgery Branch, National Cancer Institute.

4. Pulmonary disease occurring in an autopsy population of Cairo, Egypt, UAR. (Lung tissue from a year of consecutive autopsy cases is being reviewed). In collaboration with Dr. A.W. Cheever, National Institute of Allergy and Infectious Diseases.

5. Pneumocystis Carinii Pneumonia: A light and electron microscopic study of thirty National Institutes of Health, Clinical Center patients. In collaboration with Drs. D.E. Powell, Georgetown University Medical Center, and G. W. Geelhoed, formerly Surgery Branch, National Cancer Institute.

6. Open-lung biopsy in the diagnosis of pneumocystis pneumonia. In collaboration with Dr. L.L. Michaelis, Surgery Branch, National Heart and Lung Institute.

7. Post-transplant pneumonitis: A clinical-pathological study of acute pulmonary disease occurring in a series of leukemic patients following bone marrow transplantation. In collaboration with: Drs. D.E. Powell, Georgetown University Medical Center, P.V. Wooley, Medical Oncology Branch, National Cancer Institute, R.G. Graw, Pediatric Oncology Branch, National Cancer Institute, and G.R. Krueger, University of Cologne, West Germany.

8. The incidence and type of lung cancer occurring in vinyl chloride workers. In collaboration with Drs. H. Falk, Center for Disease Control, Atlanta, Georgia, L.B. Thomas, Laboratory of Pathology, National Cancer Institute, and M. Kuschner, Health Sciences Center, State University of New York, Stonybrook, New York.

9. Other Activities:

   (a) Participant, Collaborating Center, International Reference Center for Classification of Uterine and Placental Tumors.
   (b) Member, International Association for the Study of Lung Cancer.

Publications:

(a) Powell, R.D., Larson, A.L., and Henkin, R.I.: Nasal mucous membrane biopsy in Sjogren's Syndrome: A new technique for evaluation of the histologic features. Ann. Intern. Med. 81:25-31, 1974.

(b) Jones, A.E., Larson, A.L., Powell, R.D., Johnston, G.S., and Henkin, R.I.: Localization of ^{99m} Technetium in the region of the nose in Sjogren's Syndrome. Annals of Otology, Rhinology and Laryngology. Vol. 83:370, 1974.

B. Dr. Bernard L. Kasten:

1. Pathologic and microbiologic study of a case of mitral valve ring abscess due to Listeria monocytogenes. In collaboration with Drs. W.C. Roberts and Ernest Arnett, National Heart and Lung Institute.
2. Pathologic and mycologic evaluation of a case of papillary muscle mucormycosis in a patient with Waldenstroms macroglobulinemia. In collaboration with Drs. W.C. Roberts and Ernest Arnett, National Heart and Lung Institute.
3. Pathologic evaluation of a case of histoplasmosis in a porcine valve. In collaboration with Dr. Ernest Arnett, National Heart and Lung Institute.

Publications:

(a) Kagan, R.L., Brenner, V.J., and MacLowry, J.D.: Contamination of Difco bacto blood culture bottles. N. Engl. J. Med. 290:1024, 1974.

(b) Spiegel, A.M., Beazley, R.M., Kasten, B.L.: Intrathyroidal parathyroids an overlooked cause of failure in parathyroid surgery. JAMA (In Press)

C. Dr. Jerry Kolins:

1. A study on the clinico-pathological correlations in a patient with dysgerminoma of the hypothalamus. In collaboration with Drs. A.M. Spiegel and P. Gorden, National Institute of Arthritis, Metabolism, and Digestive Diseases.
2. A study of granulomatous angiitis of nervous system including attempts to isolate herpes virus and electron microscopic studies. In collaboration with Drs. R.K. Rajjoub, National Institute of Neurological Diseases and Stroke and Jose Costa, Laboratory of Pathology, National Cancer Institute.
3. A study of methotrexate leukoencephalopathy in patients with acute lymphocytic leukemia. In collaboration with Dr. P. Pizzo, National Institute of Neurological Diseases and Stroke.

4. A study of leydig cell tumor associated with corticosteroid synthesis. In collaboration with Dr. Richard Sherins, National Institute of Child Health and Development.

5. A study of Von Recklinghausen's neurofibromatosis associated with multiple pulmonary granulomas. In collaboration with Surgery Branch, National Cancer Institute.

6. A study of fatal herpes virus pneumonitis in a patient with histiocytic lymphoma.

7. A ten year review of malignant bone tumors assessing histologic subclassification.

8. A study of the pathogenesis, angiographic characteristics and treatment of experimental traumatic cerebral abscesses: Phase I experimental model under the direction of Drs. James Wood and Ayub Ommaya, National Institute of Neurological Diseases and Stroke.

D. Dr. Michael M. Orlando:

1. Clinical study of associated hyperlipoproteinemias in patients with angiographically documented coronary artery disease. In collaboration with Drs. P. Herbert and S. Epstein, National Heart and Lung Institute.

2. Study of experimental induction of viral myocarditis and cardiomyopathy in small mammals. In collaboration with Drs. S. Hajdu, National Heart and Lung Institute, Nathaniel Young, Alan Rabson and Jose Costa, National Cancer Institute.

3. Clinical study of the possible infectious etiology of idiopathic cardiac failure in patients with previous open heart surgery. In collaboration with Dr. Robert Francis, Department of Radiology, Clinical Center, National Institutes of Health.

Publications:

(a) Kwon, J., Young, R., and Orlando, M., Pulmonary mucormycosis caused by Cunninghamella elegans in a patient with chronic myelogenous leukemia: J. of Clin. Path., 1974.

E. Dr. Robert Stern:

1. Translation of Type II collagen from rat chondrosarcoma in a cell-free system derived from wheat germ. In collaboration with J. Wilczek, National Institute of Dental Research.

2. Messenger RNA dependent cell-free synthesis of collagen: Hydroxylation of the reaction product. In collaboration with A. Ruggieri and K. Benveniste, National Institute of Dental Research.

3. Translation of collagen mRNA from chick embryo calvaria in a cell-free system derived from wheat germ. In collaboration with K. Benveniste and J. Wilczek, National Institute of Dental Research.

4. Homogeneity of pilocarpine preparations. In collaboration with Dr. D.A. Newsome, formerly of National Eye Institute presently with Massachusetts General Hospital.

5. Collagen biosynthesis in the Schistosomiasis-induced hepatic granuloma. In collaboration with Dr. A.W. Cheever, National Institute of Allergy and Infectious Diseases.

6. Isolation and characterization of collagen mRNAs from various sources. In collaboration with Drs. Diaz deLeon and Breitkreutz, Visiting Scientists, National Institute of Dental Research.

7. Preparation of immune sera directed against the various species of procollagen molecules.

8. Translational control mechanisms for collagen biosynthesis.

9. Survey of hepatitis B_s Ag positive patients, using Clinical Center (1970-1975) surgical and autopsy material. This study will evaluate the orcein staining reaction as an epidemiological tool and as a clinical correlate in the antigen positive patient. In collaboration with Dr. Nathaniel Young, National Cancer Institute.

Publications:

(a) Stern, R., Rose, J.A., and Friedman, R.M.: Phleomycin Inducted Cleavage of DNA: Biochemistry 13:307, 1974.

(b) Friedman, R.M., Stern, R., and Rose, J.A.: Phleomycin Stimulation of Thymidine Triphosphate Incorporation by Animal Cell Nuclei: J. Nat. Cancer Inst. 52:693, 1974.

(c) Newsome, D.A., and Stern, R.: Pilocarpine Adsorption by Serum and Ocular tissues: Am. J. of Ophthalmology 77:918, 1974.

F. Dr. Maria M. Tomaszewski:

1. Review and evaluation of pathology of breast cancer cases for the Breast Tumor Service. In collaboration with Dr. D.E. Henson, National Cancer Institute.

2. Histopathologic evaluation of morphology of breast cancer in patients 35 years of age and less and 65 years or older. In collaboration with Dr. D.E. Henson, National Cancer Institute.

3. Case report of primary bronchogenic carcinoma. In collaboration with Drs. M. Cohen and P.T. Scardino, Surgery Branch, National Cancer Institute.

4. Review of pathology of intralesional treatment of malignant melanoma. In collaboration with Drs. A.S. Rabson, Laboratory of Pathology and M. Cohen, Surgery Branch, National Cancer Institute.

Publications:

(a) D.E. Henson, G.W. Geelhoed, and M.M. Tomaszewski: Benign and Second Malignant Primary Neoplasms in Women Dying of Breast Cancer Versus Lymphoma. (In Press)

Keyword Descriptors: anatomic pathology/ surgical pathology/ pathology residency training.

Project No. Z01 CBD 00582-04 LP
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pathogenesis of Chronic Mouse Cytomegalovirus Infection in Lymphoid Organs and Effect of Chronic Infection on Development of Lymphoma

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Margery Sullivan; and Duran Harris

Other Investigators: A.J. Strano

Cooperating Units: Armed Forces Institute of Pathology

Man Years:

Total: 1
Professional:
Others:

Project Description:

Objectives: Study the mechanisms of latent murine cytomegalovirus infection in the salivary glands and lymphoid tissue of mice chronically infected with this virus and determine effect of chronic infection on lymphoma development.

Methods Employed: Tissue culture, immunofluorescence, electron microscopy.

Significance: (NCP Objective 1, Approach 4) The mouse cytomegalovirus causes chronic infection in the submaxillary glands, lymph nodes, and spleens of many strains of mice. Infection in the lymph nodes and spleen probably occurs in lymphocytes. The murine cytomegalovirus belongs to the herpesvirus group of animal viruses, along with the Epstein-Barr virus and *Herpesvirus saimiri*.

Proposed Course: This work is completed. Results indicate that chronic CMV infection does not enhance or accelerate natural development of lymphoma in mice.

Keyword Descriptors:

Virology, lymphoma, mouse cytomegalovirus, chronic viral infection, latent viral infection, cancer, animal models

Publications:

1. Henson, D.E., Grimley, P.M., and Strano, A.J.: Postnatal cytomegalovirus hepatitis. Hum. Pathol. 5: 93-103, 1974.

Project No. Z01 CBD 00583-02 LP
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunological, Biological, and Ultrastructural Studies of Surface Antigens on Leucocytes and Other Tumor Cells

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Ralph Helmsen, Ph.D.; and Duran Harris

Other Investigators: None

Cooperating Units: National Eye Institute

Man Years:

Total: 1
Professional: 1/4
Others: 3/4

Project Description:

Objectives: 1. Develop immunologic and ultrastructural techniques for examination of surface antigens and receptor sites on human leucocytes and on tumor cells.

2. Study effects of overt and covert infections of viruses on the distribution and types of surface receptors on leucocytes and other cells.

Major Findings: Hybrid antibody labeling technique has been developed to localize viral antigens in cultured cells.

Significance: (NCP Objective 3, Approach 5) Development of immunologic and ultrastructural techniques will aid in our understanding on the distribution of surface antigens, leucocytes, and tumor cells.

Proposed Course: Hybrid antibody method of immunolabeling for electron microscopy has been developed in our laboratory. System will be applied to tumor antigens and to virus infected cells.

Keyword Descriptors:

Hybrid antibody, electron microscopy, viruses, cancer, tumor cells, cell surface antigens

Publications:

1. Henson, D.E., Helmsen, R., Becker, K.E., Strano, A.J., Sullivan, M., and Harris, D.: Ultrastructural localization of herpes simplex virus antigens on rabbit corneal cells using sheep antihuman IgG antihorse ferritin hybrid antibodies. *Invest. Ophthalmol*. 13: 819-827, 1974.

Project No. Z01 CBD 00585-02 LP
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Histological Evolution of In-Situ Carcinoma in the Vagina

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; and Glenn W. Geelhoed, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1/4
Professional: 1/4
Others:

Project Description:

Objectives: Clinical-pathological study of women with multicentric squamous cell cancers in the ectogenital epithelium.

Major Findings: Histological studies made on women with sequential vaginal biopsies have demonstrated progression from severe epithelial atypia, through dysplasia, to carcinoma in-situ. Like carcinoma of the cervix, in-situ vaginal tumors have an invasive potential. In-situ vaginal tumors associated with carcinoma of the cervix seem to form a distinctive clinical syndrome.

Significance: (NCP Objective 5, Approach 6) Many women with cancer of the uterine cervix subsequently develop epithelial cancers in the vagina and/or vulva. These multiple cancers have been considered to arise in an epithelial region predisposed to malignancy. This region has a common cloacal origin embryologically.

Proposed Course: Project is still in effect. Patients will be added to the study as they are diagnosed. A report has been submitted for publication.

Keyword Descriptors:

In-situ carcinoma, in-situ cancer, vaginal carcinoma, cancer, cervical cancer, dysplasia, multicentric cancer, non-invasive cancer, female cancer

Project No. Z01 CBD 00590-02 LP
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Histopathological Studies of Breast Cancer

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Maria Tomaszewski, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1/2
Professional: 1/2
Others:

Project Description:

Objectives: 1. Attempt to relate histopathological findings on breast cancer with prognosis, efficacy of therapy, and other diseases.

2. Attempt to define new classifications of breast cancer.

3. Relate histology of breast cancer to various serum tumor markers, such as CEA.

Major Findings: Results indicate that patients with breast cancer have a different spectrum of second benign and malignant tumors than female patients with lymphoma. Also found that breast epithelial cells contain a myosin-like protein.

Significance: (NCP Objective 5B, Approach 4) Breast cancer is a leading cause of death in women in the United States. The significance of these studies is to use histopathological data to guide therapy and predict prognosis.

Proposed Course: Continue to study and to classify breast cancer cases at NIH and to associate histological observations with clinical and laboratory observations.

Keyword Descriptors:

Breast cancer, mammary cancer, histology of breast cancer

Project No. Z01 CBD 00591-01 LP
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunofluorescence Studies on Human Tumors

Previous Serial Number: None

Principal Investigator: Donald E. Henson, M.D.

Other Investigators: R. Levinson and D. Harris

Cooperating Units: Laboratory of Biochemistry, NHLI

Man Years:

Total: 1
Professional: 1/5
Others: 4/5

Project Description:

Objectives: Use of immunofluorescence on frozen section material to look for novel or unusual proteins synthesized by human tumors.

Methods Employed: Immunofluorescence immunology

Major Findings: Dedifferentiated rhabdomyosarcoma cells produce a new type of myosin immunologically related to myosin present in fibroblasts.

Significance: (NCP Objective 4, Approach 2) The purpose is to search for novel proteins in tumors and to determine if these proteins can be useful in the early diagnosis of cancer, diagnosis of precancerous lesions, and for predicting prognosis.

Proposed Course: Test a variety of tumors by immunofluorescence using antibodies prepared against purified proteins that have been isolated from fetal tissue, normal organs, or from other sources.

Keyword Descriptors:

Immunofluorescence, tumor antigens, antibodies, cancer diagnosis, cancer

Project No. Z01 CBD 00592-01 LP
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Freeze Etch Electron Microscopic Examination of Virus Induced Membrane Receptors

Previous Serial Number: None

Principal Investigator: Timothy J. Triche, M.D., Ph.D.

Other Investigators: J.C. Costa

Cooperating Units:

Man Years:

Total: 1
Professional: 1/2
Others: 1/2

Project Description:

Objectives: 1. To characterize the membrane antigen (MA) of herpes simplex with regard to the time of appearance following infection, the distribution, and the association with lectin receptor sites, in cultured retinoblastoma cells.

2. To compare these results with those in the literature, and to determine the association, if any, of these membrane sites with the increased agglutinability of transformed cells by the lectin concanavalin A.

Methods Employed: 1. Freeze etch electron microscopy, utilizing high resolution platinum-carbon replicas of fractured and etched cell surfaces examined in the transmission electron microscope.

2. Labelling of these surfaces with ferritin or hemocyanin-tagged lectin or immunoglobulin.

Significance: (NCP Objective 3, Approach 5) 1. The known increased agglutinability of transformed cells by plant lectins (such as concanavalin A) may be related to an alteration in number or distribution of cell surface receptors for lectins, which may coincide with the appearance of viral-associated material in the membrane.

2. A double label study, labelling both viral antigen-associated membrane sites and lectin binding sites, should provide unequivocal evidence, pro or con, of the means whereby viruses induce increased cell agglutinability, a property of cells associated with oncogenesis.

Proposed Course: Considerable time will be spent preparing reagents for labelling the membranes as well as installing the freeze etch device. Once the technological aspects are assimilated, the project will progress forthrightly through preliminary experiments and finally through repetitive testing to establish reproducibility. Later, other parameters, such as cell type, virus type, temperature, type of lectin, etc. will be evaluated.

Keyword Descriptors:

Freeze etch electron microscopy, viral-induced membrane antigens, membrane receptors for plant agglutinins, agglutinability of transformed cells, oncogenesis

Publications:

1. Triche, T.J., Tillack, T.W., and Kornfeld, S.: Localization of receptor sites for *Ricinus communis* and *Agaricus bisporus* lectins on human erythrocyte membranes. Amer. Assoc. Path. Bact., March 9-12, 1974, pp. 86a-87a.

Project No. Z01 CBD 00531-06 LP
1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In Vitro Studies of Human Malignant Tumors and Oncogenic Viruses

Previous Serial Number: Consolidation of 531 and 532

Principal Investigator: Alan S. Rabson, M.D.

Other Investigators: J. Costa, E.W. Chu, D. Albert (Yale), S. Rosen (NIAMDD) and K. Takemoto (NIAID)

Cooperating Units:

Man Years:

Total: 1
Professional: 2/3
Others: 1/3

Project Description:

Objectives: To study biological properties of human lymphoma, sarcoma, carcinoma and retinoblastoma cells *in vitro* and to attempt to isolate oncogenic viruses from these cells.

Methods Employed: Standard tissue culture, virologic and pathologic techniques are used.

Major Findings: 1. ChaGo cell line: Studies of this cell line derived from a human lung cancer which produce human chorionic gonadotropin *in vitro* have continued. A clonal line derived by Dr. Kim in our laboratory has been found to produce very high levels of the alpha subunit of hCG. In collaboration with Dr. Rosen's associates in NIAMDD (Drs. Lieblich and Weintraub) we have studied differences between ectopic and non-ectopic secretion of hCG and its subunits by comparing our clone from ChaGo with clonal lines from trophoblastic tumors derived by Dr. Peter Kohler. Under identical cultural conditions, secretion rates in picomoles day^{-1} (mg protein)$^{-1}$ were:

ChaGo:	hCG less than 0.01 alpha 160-190 beta less than 1	Trophoblastic tumor (JEG-3)	hCG 8-18 alpha less than 1.0 beta less than 1.0

These results imply major differences in control of ectopic versus non-ectopic production of hCG. Moreover, the high alpha secretion of the ChaGo clone suggests that in certain cases ectopic protein production may be even more efficient than non-ectopic production.

2. Studies of retinoblastoma cells have continued in collaboration with Dr. Albert and Dr. Char. Fourteen patients with retinoblastoma, 5 normal volunteers and 6 other patients were tested for cell-mediated immunity by the ^{125}I-iododeoxyuridine cytotoxicity assay against our retinoblastoma cell line and as a control against a breast cancer cell line. Eleven of the 14 patients with retinoblastoma had significantly increased cytotoxicity against the retinoblastoma cell line.

3. Studies of the human papovavirus isolated from the brain tumor of the Wiscott-Aldrich patient have continued in collaboration with Dr. Takemoto. The isolate has been shown to be serologically similar to the BK virus, and serum from the patient showed development of a high antiviral antibody at least 1 year before the development of the tumor.

4. Herpesviruses: See annual report of Dr. J. Costa (00533)

Significance: (NCP Objective 3, Approach 2) 1. If it can be shown that the human papovavirus we have isolated from the reticulum cell sarcoma of the brain of the Wiscott-Aldrich patient is etiologically related to the tumor, it will be the first example of a human malignant tumor produced by a virus.

2. Studies of ectopic hormone production by the human lung cancer cell line will give us new insight into mechanisms and significance of ectopic hormone production by human cancer cells and may give information of the nature of derepression associated with malignant transformation.

3. The possibility that human retinoblastomas are induced by viruses remains a reasonable hypothesis and the evidence of cell-mediated immunity against these tumors supports this. The demonstration of the cell-mediated immunity also suggests that methods to increase this immunity might be of value in therapy.

Proposed Course: Attempts to characterize the human papovavirus isolated from the brain tumor and to establish its role in the etiology of the tumor will continue. Studies of the factors involved in ectopic gonadotropin secretion by lung cancers will continue. Studies of the possible viral etiology of human retinoblastoma will also continue.

Keyword Descriptors:

Tissue culture, viruses, ectopic hormones, human chorionic gonadotropin, retinoblastoma, papovaviruses, Wiscott-Aldrich syndrome, cell-mediated immunity.

Publications:

1. Buja, L.M., Ferrans, V.J., and Rabson, A.S.: Unusual nuclear alterations. Lancet I: No. 7854, 402-403, 1974.

2. Char, D.H., Bergsma, D., Rabson, A.S., Albert, D.M., and Herberman, R.B.: Cell-mediated immunity to retinal antigens in patients with pigmentary retinal degenerations. Invest. Ophthalmol. 13: 198-203, 1974.

3. Bastian, F.O., Rabson, A.S., Yee, C.L., and Tralka, T.S.: Herpesvirus varicellae isolated from human dorsal root ganglia. Arch. Pathol. 97: 331-333, 1974.

4. Levinson, W., Coleman, V., Woodson, B., Rabson, A., Lanier, J., Whitcher, J., and Dawson, C.: Inactivation of herpes simplex virus by thiosemicarbazones and certain cations. Antimicrob. Agents Chemother. 5: 398-402, 1974.

5. Lewis, A.M., Jr., Rabson, A.S., and Levine, A.S.: Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. X. Transformation of hamster kidney cells by adenovirus 2 and the nondefective hybrid viruses. J. Virol. 13: 1291-1301, 1974.

6. Costa, J.C., Rabson, A.S., Tralka, T.S., Engel, W.K., Canellos, G.P., and Bratenahl, C.G.: Leukemia and lower-motor-neuron disease. Lancet II: No. 7872, 107-108, 1974.

7. Ludueña, M.A., Sussman, H.H., and Rabson, A.S.: Synthesis of human placental alkaline phosphatase in vitro by the ChaGo cell line. J. Natl. Cancer Inst. 52: 1705-1709, 1974.

8. Reid, T.W., Albert, D.M., Rabson, A.S., Russell, P., Craft, J., Chu, E.W., Tralka, T.S., and Wilcox, J.L.: Characteristics of an established cell line of retinoblastoma. J. Natl. Cancer Inst. 53: 347-360, 1974.

9. Char, D.H., Ellsworth, R., Rabson, A.S., Albert, D.M., and Herberman, R.B.: Cell-mediated immunity to a retinoblastoma tissue culture line in patients with retinoblastoma. Am. J. Ophthalmol. 78: 5-11, 1974.

10. Takemoto, K.K., Rabson, A.S., Mullarkey, M.F., Blaese, R.M., Garon, C.F., and Nelson, D.: Isolation of papovavirus from brain tumor and urine of a patient with Wiskott-Aldrich syndrome. J. Natl. Cancer Inst. 53: 1205-1207, 1974.

11. Costa, J., Yee, C., Troost, T., and Rabson, A.S.: Effect of dexamethasone on herpes simplex virus type 2 infection in vitro. Nature 252: No. 5485, 745-746, 1974.

12. Costa, J.C., and Rabson, A.S.: Role of Fc receptors in herpes simplex virus infection. Lancet I: No. 7898, 77-78, 1975.

13. Rabson, A.S., and Costa, J.C.: Viruses and the etiology of human hematopoietic neoplasms. Int. Acad. Pathol. Monogr. In Press.

14. Muñoz, N., Aristizabal, N., Yee, C., Rabson, A., Pearson, G., and de-Thé, G.: Antibodies to herpesvirus in patients with cervical cancer. Monograph (IARC) In Press.

15. Tralka, T.S., Rabson, A.S., and Hansen, C.T.: C-type virus particles in a cell line from a lymphosarcoma of a "nude" mouse. J. Natl. Cancer Inst. In Press.

16. Didier, M.L., Ablashi, D.V., Oie, H.K., Armstrong, G.R., Chu, E., and Rabson, A.: Some biological properties of Herpesvirus saimiri (HSV) from chronically infected monolayer and suspension cultures. Monograph (IARC) In Press.

Project No. Z01 CBD 00533-02 LP
1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Oncogenic DNA Viruses

Previous Serial Number: None

Principal Investigator: Jose C. Costa, M.D.

Other Investigators: A.S. Rabson, N.A. Young, and C.J. Herman

Cooperating Units:

Man Years:

Total: 1
Professional: 2/3
Others: 1/3

Project Description:

Objectives: 1. To study biological properties of human herpesviruses and human papovaviruses tentatively associated with tumors in human patients.

2. To study lymphocyte blastogenic response of human patients to viral antigens (herpes simplex and papovaviruses).

Methods Employed: Standard tissue culture, virologic and pathologic techniques are used. Rapid flow microfluorometer is used to evaluate lymphocyte blastogenic response.

Major Findings: 1. Rapid flow microfluorometer measurement of the cell DNA content has been applied to determine lymphocyte blastogenic response to PHA and a specific antigen (PPD). Work is now in progress to apply this assay to evaluate blastogenic response of human peripheral blood lymphocytes to viral antigens.

2. Two of eleven Syrian hamsters inoculated by intracerebral route with M.M. virus (a human papovavirus isolated from the brain of a patient with Wiscott-Aldrich syndrome) have developed intracranial tumors 6 months post-injection.

3. Studies are in progress to elucidate the possible biological significance of a receptor-site for the Fc fragment of immunoglobulin that appears in herpes simplex infected cells. It has been found that engagement of the Fc site significantly decreases the total yield of virus from infected cells.

Significance: (NCP Objective 3, Approach 2) 1. The methodology developed may provide an accurate way to assess cellular immunity on humans to a variety of antigens, and may help in gathering evidence to implicate human herpesviruses and human papovaviruses on the etiology of human neoplasia.

2. Since the human papovavirus was isolated from a brain tumor, it will be of interest to study the CNS tumors caused in hamsters by I.C. inoculation.

3. Experiments are being performed to test if molecules of immunoglobulin, when bound to the Fc receptor, can mask viral antigenic sites on the cell surface, thus acting as blocking antibodies.

4. Proof that an Fc receptor site may be the basis for a blocking antibody mechanism may provide further understanding of the physiopathology of the herpesvirus infection and may also serve as a model to understand the significance of Fc receptor sites on tumor cells.

Proposed Course: 1. Preparations of herpesvirus virions (capsids versus enveloped virus) are being purified in order to be tested as antigens in a lymphocyte transformation assay.

2. Experiments are in progress to test the oncogenic potential of M.M. virus in newborn hamsters using the intravenous route for inoculation of the agent.

3. Attempts to establish cell lines from a variety of human brain tumors obtained at surgery will continue. These lines may provide a suitable approach for evaluating the role of human papovaviruses in CNS neoplasms.

Keyword Descriptors:

Tissue culture, viruses, cell-mediated immunity

Publications:

1. Costa, J., Yee, C., Troost, T., and Rabson, A.S.: Effect of dexamethasone on herpes simplex virus type 2 infection in vitro. Nature 252: No. 5485, 745-746, 1974.

2. Costa, J.C., and Rabson, A.S.: Role of Fc receptors in herpes simplex virus infection. Lancet I: No. 7898, 77-78, 1975.

Project No. Z01 CBD 00534-01 LP
1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structure and Assembly of Virulent and Vaccine Polioviruses

Previous Serial Number: None

Principal Investigator: Nathaniel A. Young, M.D.

Other Investigators: J. Nakano, B. Elisberg, and R. Moon

Cooperating Units: Enterovirus Unit, Center for Disease Control; Food and Drug Administration; Michigan State University

Man Years:

Total: 1 1/4
Professional: 2/3
Others: 3/4

Project Description:

Objectives: To study molecular correlates of diminished neurovirulence in Sabin vaccine strains of polioviruses by comparing their structure and assembly with wild type polioviruses.

Methods Employed: Standard techniques of virology, tissue culture, gradient centrifugation, gel electrophoresis, and nucleic acid hybridization.

Major Findings: The nucleotide sequences of RNA from type 1 poliovirus vaccine and the parental neurovirulent strain differ by about 5%. The largest virion polypeptide, VP1, of the vaccine is structurally labile and differs slightly in size from the corresponding protein in the virulent strain.

Significance: (NCP Objective 3, Approach 2 and NCP Objective 2, Approach 1) Structural lability and size differences in vaccine polypeptides may alter the conformation of the assembled virion, thereby impairing its attachment to specific receptors on nerve cells. This postulated mechanism may explain diminished virulence of the vaccine strains. Ultimately candidate vaccines directed against oncogenic and other viruses may be selected on the basis of alterations in the affinity of virion polypeptides for target cells.

Proposed Course: The significance of the alterations in attenuated type 1 poliovirus will be assessed by determining whether similar molecular lesions exist in the vaccine strains of antigenic types 2 and 3. The strains would lend support to the hypothesis that a causal relationship exists between alterations in capsid architecture and diminished neurovirulence. As a spin off of the above findings, the potential use of analysis of viral proteins as a marker for vaccine strains will be explored. Strains of poliovirus isolated from human vaccine-associated paralytic disease, provided by the FDA and the Center for Disease Control, will be examined by these techniques and the results correlated with other biological markers.

Keyword Descriptors:

Viruses, vaccines, virulence, RNA, hybridization, polypeptides, gel electrophoresis

Annual Report of the Image Processing Unit, Diagnosis Branch, Division of Cancer Biology and Diagnosis
National Cancer Institute

The Image Processing Unit was transferred from the Division of Cancer Treatment to the Division of Cancer Biology and Diagnosis on July 1, 1974. This had been agreed to by the then respective Division Directors, Drs. Zubrod and Berlin, two years previously at the time of initial activation of the Unit - July 1972.

The completion, essentially on schedule, of the breadboard model Grain Counter (Project No. Z01 CBD 08475-03 IMP) and its repeatedly demonstrated functional state, marked the approximate break-point in the first half of calendar 1974. The remaining obligation to the Division of Cancer Treatment, i.e. redesign, replication and introduction of working devices into the field, was essentially a service and only secondarily a research activity. It had been agreed prior to July 1974 that DCT would support procurement and construction by a competitively selected vendor of four systems for field service, as well as bringing the existing Grain Counter into mechanical and electronic identity with the to be constructed field models.

The redesign, debugging and reconstruction of the first model Grain Counter was completed during the reporting period. The various procedures for competitive selection of a vendor have been completed and the results of technical and financial evaluation of all submitted proposals are in the hands of the Procurement Section at this writing; construction of the four field models awaits completion of negotiations by Procurement with the selected vendor.

The Grain Counter/Densitometer (GC1.1) was originally intended to go beyond simple counting of autoradiographic grains in microscopic images. Its present functioning capabilities include the ability to automatically separate grains for each cell into nuclear and non-nuclear categories, to measure the area of the nucleus of each cell (to about 3 percent precision) and its optical density (to about 6 percent precision). Individual results on running totals for each of the 16 user assignable classes of cells are kept automatically. A variety of shape related measures are also obtainable. By changing one or more filters in the array of six computer controlled filter changers, it is possible to quantitate cytoplasm, various inclusions and/or extracellular components. Additionally, the Grain Counter may function as an "electronic micrometer," a thickness measuring device (focus is also under remote/computer control), etc. The Grain Counter "remembers" the position of each cell measured and may return to each such user on command or via a variety of automatic sequences. It is characterized by a variety of user convenience programs which makes operation by technicians with little or no knowledge of computers simple and direct. It is further characterized by a variety of "self-protective" mechanisms which prevent inadvertent damage by a careless user.

Without any additional hardware, it is possible by simple program changes (by the naive user) to convert the instrument into a simple "shape"/ texture analyzer (this is now functional), an analyzer of double labeled material (provision in system software exists for necessary subroutines to be written), or a cell locator (exists and functional).

Provision for additional expansion of capabilities along lines required by individual users exists. Additional functions (e.g. the ability to delineate irregularly shaped regions for analysis by "drawing" outlines on the screen) may be embodied on a circuit card for direct plug-in to the existing electronics without redesign or rewiring.

In redesign of the Grain Counter, the Image Processing Unit relied heavily on the design contract with Carman Electronics, as well as on the fabrication contract with CAMBION (Cambridge Thermionic Corporation). At the same time a major portion of four staff professional's time was devoted to reprogramming and optimization of software, optics and related electrical equipment.

At the close of the reporting period, substantive studies employing the Grain Counter/Densitometer involving measurements of various cell cycle parameters in particular in L1210 leukemia cultures, but also in cultured macrophages supplied by Drs. Wunderlich and Koren ($P388D_1$) were in progress.

Designs are complete for the experimental studies for evaluation of precision, reproducibility, etc. of the field models. These collaborative experiments must of course await installation of the field models.

The other major project of the Image Processing Unit for the reporting period has been the continued work on the Automatic Cell Analysis project (Project No. Z01 CBD 08476-03 IMP). This project involves the construction of 1) a fully computer controlled microscope, a special purpose processor and a controlling mini-computer along with necessary monitor and debugging software, and 2) an artificial intelligence-like system resident in the DCRT PDP-10, embodying a procedural description language and facilities for on-line modeling of cell descriptions and control facilities for group 1. The components subsumed under 1) are collectively termed the Real Time Picture Processor (RTPP) while the overall system including the higher level structures on the PDP-10 is termed the CELMOD.

This complex has been the subject of several publications appearing during the reporting period as well as extensive detailed documentation (manuals running several hundred pages each which are scheduled for eventual publication by the Clearinghouse for Technical Information).

The first cell material which the system will be applied is normal and pathologic bone marrow smears. In order to design a hardware/software system to accomplish such an automated procedure, cell recognition algorithms must be interactively specified and tested by the biologist. The RTPP/CELMOD system is the test bed in which this is and will continue to take place. An eliciting of a biologist's knowledge concerning cell image classification and description requires considerable specialized computing capability working at more or less real time rates. It is for this reason that so much computing power must be built into the local device, the RTPP.

Work on the RTPP in collaboration with Carman Electronics and Carl Zeiss has progressed rapidly during the reporting period. Accomplishments include:

a) The installation of the Axiomat Microscope and its interfacing to the PDP8 process control component.

b) Installation of the dual scanning system on the Axiomat and the attainment of full computer control of the mirror and the plumbicon scanners.

c) Integration and interfacing of the Quantimet 720 special purpose image acquisition/analysis subsystem into the RTPP complex.

d) Construction of full control facilities for biologist use of the RTPP (both present and future functions).

e) Construction of the mask register component which allows hardware logical operations on image and subparts of images.

f) Integration and interfacing of the sonic digitizing tablet which will allow biologists to point or otherwise delineate objects within a microscopic field for measurement or other image processing use.

g) Construction of a software monitor debugging system which allows operation of current level of RTPP and allows control (user or computer) of each component as installed and integrated into the system.

Work on the procedural description language has accelerated since the decision to implement all PDP-10 material in the Stanford Artificial Intelligence Language (SAIL). At the conclusion of the reporting period, an integral component of the procedural description language, a "parser generator," was completed and operational.

Other Activities:

The RTPP/CELMOD system is intended to perform real time measurements and characterization of living cells as well as fixed and stained material. In fact the ability to track a cell and its direct descendants through several successive cell cycles is a major reason for constructing a system of such complexity.

During the reporting period, a modest beginning was made toward the specification of a computer controlled stage with both the speed and precision necessary to perform such operations in real time. A set of rectified objectives for polarized light observation (one of the three or four such in existence) was acquired for polarized light measurements of microtubules and microfilaments in living cells. This equipment is currently being used for this purpose by Dr. Robert Tucker of Dr. Sanford's section. As the RTPP becomes operational, these measurements will be transferred to this equipment. An air curtain incubator has been incorporated in the system and has been found compatible with the measuring apparatus.

Because automatic chemical environmental control is part of the living cell monitoring problem, we regard the time spent on the general review of computers in the clinico-pathologic lab, chemistry and image processing to be particularly well spent since at its completion we are now fully capable of specifying such an environmental control system.

Personnel:

Bruce Shapiro, formerly a consultant to Arthur D. Little, has been employed as an Expert Consultant in Computer Science. Gerson Grosfeld has been employed as a Computer Programmer. Thomas Devoe has been given a temporary appointment as an Electronic Technician.

The last appointment is only incomplete and temporary relief of our most pressing need for electronic technician assistance.

Space:

During the reporting period, plans were begun for the physical move of the Unit to space in the Frederick National Cancer Center.

Project No. Z01 CBD 08475-03 IMP
1. Diagnosis Branch
2. Image Processing Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Automated Grain Counting/Densitometer

Previous Serial Number: NCI-7070

Principal Investigators: Lewis E. Lipkin
Peter F. Lemkin

Other Investigators: Morton L. Schultz
George M. Carman
Marta Jo Wade
Bernice S. Lipkin
Gerson Grosfeld

Cooperating Units: Computer Center Branch, DCRT
Biomedical Engineering and Instrumentation Branch, DRS
Carman Electronics, Corvallis, Oregon

Man Years:

Total:	5.5
Professional:	2.5
Others:	3.0

Project Description:

The intent of Drs. Perry and Zubrod (then of DCT) in providing the original mandate to the Image Processing Unit was to create instrumentation for semiautomatic to automatic measurement of various cellular parameters (especially those of interest to cell kinetics and particularly tritiated thymidine uptake) in microscopic images. Their direction and the plan followed subsequently was to 1) construct (as far as possible from off-the-shelf components) first a reasonably flexible device for automatic measurements of user selected cells, and 2) concurrently and subsequently develop hardware and software constructs directed toward the eventual full automation of a still broader series of measurements applicable to a wider range of histologic and cytologic material, making use where possible of existing stained slides.

The design and prototype construction of the first objective, the Grain Counter/Densitometer (GC1.1), is now accomplished. The device which has emerged consists of a 32K PDP8/e Computer, a Metals Research Quantimet 720, a Zeiss Universal Research Microscope, a specially designed control desk, and relating the others to one another and the user, the specialized electronics generally termed the interface. The Image Processing Unit is now completing arrangements for production of four such instruments to be placed for field trial at the following institutions:

1. Brookhaven National Laboratory - Dr. Eugene Cronkite
2. Memorial Sloan-Kettering Cancer Center - Dr. Bayard Clarkson
3. Allegheny General Hospital - Dr. Lewis Schiffer
4. Southern Research Institute - Drs. H. Skipper and L. Simpson-Herren

These arrangements have occupied a major portion of the time and effort of the Unit during the reporting period. They included: 1) Conversion of the working but "breadboard" model into a form which is sufficiently simple and compact to allow day-to-day trouble-free field operation. This redesign accomplished in conjunction with Carman Electronics is now complete, physically embodied, operational, debugged and fully documented down to individual circuit pin assignments. In the process, the more than 100 low density circuit cards of the breadboard have been replaced by equivalent circuitry on only five large custom made circuit boards. 2) Again, in conjunction with Carman Electronics, a series of test programs which may be used in the field for troubleshooting the system have also been developed, tested and incorporated into the software system. In case of difficulty, these programs will inform the user which of the circuit cards is malfunctioning. Since the instruments when placed in the field will be accompanied by a complete duplicate set of cards, the instruments for the most part should be subject to easy first-line repair and/or maintenance. 3) Arrangements for construction of the necessary cards, their duplicates for the field models, a computer checked wire listing, and checking for logical inconsistency has been accomplished with a contract with Cambridge Thermionic Corporation. It is estimated that were this work (now complete) to be hand done, it would have required three man years of technician time, a resource which is desperately short. 4) The extremely time-consuming and laborious process of minutely specifying the components and construction details for the field model has been completed. Last year's sources sought solicitation was followed by a formal request for proposal. The technical and financial responses have been evaluated at this writing and we are awaiting the completion of negotiations before announcement of the award of contract. 5) The (200 pages plus) prototype users manual was revised by us for use during the four one-week user familiarization sessions planned for the first quarter of FY 1976. Thus, while construction of the field models is in progress, our users will learn details of machine use employing the Grain Counter now in operation in our laboratory. 6) A collection of representative autoradiographs has been gathered to be used in the "round robin" field trials of the instrument. The unique ability of the machine to recall individual cells will allow an order of magnitude increase in the precision of evaluation, since we can insure that each group will run its measurements on exactly the same cells as the others. The instrument now operational in our laboratory will participate in the field trials as a fifth machine. In conjunction we have developed a statistically valid experimental design for the trials and provision for the appropriate analyses of variance.

The first set of field trials will be for grain counting precision, while subsequent field experiments will be concerned with measures of nuclear area, density, etc.

During the reporting period a moderately detailed description of the system appeared in the Journal of Histochemistry. Another publication reported on the use of the instrument in obtaining texture measures for possible subsequent use as parameters for cell classification. Two presentations (scheduled for Asilomar in June) of results obtained with it on the cell cycle and textural parameters of L1210 leukemia had to be withdrawn because of travel restrictions. They are, however, being prepared for publication.

We have made the automatic measurement facility, Grain Counter/Densitometer, available to several investigators. One, Dr. Arthur Frank, Lung Cancer Branch, DCCP, has employed the Grain Counter to quantitate nuclear changes in tracheal organ cultures exposed to asbestos. He has reported his results in the Proceedings of the American Association for Cancer Research. Other such studies included work with Dr. Evelyn Parker, Dermatology Branch, DCBD, on grain counts in xeroderma pigmentosum.

Substantive work within the Image Processing Unit employing the Grain Counter has concentrated on two tissue culture systems. One of these, a macrophage culture ($P388D_1$) has been provided us by Drs. John Wunderlich and Hillel Koren, Immunology Branch, DCBD. At this writing, we are in the process of characterizing the cell cycle parameters of the culture employing the Grain Counter, as well as auxiliary time lapse cinematographic equipment, incidentally providing immunology with some necessary data which would not be as available to them.

The other culture of primary interst is L1210 leukemia. We are working intensively on two strains, one provided by Dr. Philip Thayer, Arthur D. Little, Inc., and the other employed by Dr. Kurt Kohn, Laboratory of Molecular Pharmacology, DCT. Large volumes of grain counts, densitometric and nuclear volume measurements will be submitted for publication during FY 1976.

Keyword Descriptors: Autoradiography
Scanner
Densitometry
Plumbicon
Light Microscopy
Cell Kinetics
Tissue Culture
Mini-Computer
Tritiated Thymidine
Blood and Bone Marrow

Honors and Awards: None

Publications:

Lipkin, L., Lemkin, P. and Carman, G.: Automated autoradiographic grain counting in human determined context. J. Histochem. Cytochem. 22(7): 755-765, 1974.

Lipkin, B. and Lipkin, L.: Textural parameters related to nuclear maturation in the granulocytic leukocytic series. J. Histochem. Cytochem. 22(7): 583-593, 1974.

4

Project No. Z01 CBD 08476-03 IMP
1. Diagnosis Branch
2. Image Processing Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Automatic Cell Analysis

Previous Serial Number: NCI-7071

Principal Investigators: Lewis E. Lipkin
Peter F. Lemkin

Other Investigators: Morton L. Schultz
Bernice S. Lipkin
George M. Carman
Sprague Hazard
Bruce Shapiro
Peter Kaiser
Edward Cook

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS
Computer Center Branch, DCRT
Carman Electronics, Corvallis, Oregon
Arthur D. Little, Cambridge, Massachusetts
Cambridge Thermionic Corporation, Cambridge, Massachusetts
Carl Zeiss, Inc., New York, New York

Man Years:

Total:	4.0
Professional:	3.5
Others:	0.5

Project Description:

The research objective is the construction of an interactive advanced designer, a general purpose device which will allow the on-line investigation of the morphological properties of classes of related biologic objects such as the cells in a marrow smear. The object of such investigation is to identify and isolate members of the optimal set of properties and morphologic relationships which,when themselves properly articulated,will allow the automatic classification of members of the various classes, their quantitation and characterization.

It is our contention that such an interactive system requires considerable specialized computing capability at more or less real time rates. The biologist in working with "real" material must be able to continuously evaluate the consequences of applying newly constructed algorithms, not only to a grain cell, but also to debris, other cells of different classes, etc. On real slides cells touch and overlap, boundaries are unclear and different tasks require different depths of subarticulation from the same image.

A major difficulty, one which we believe we are well on the way to solving, (as evidenced by the considerable progress noted below) is the construction of a very powerful system that is at the same time easy to use and which is both fast and reliable.

The other major difficulty, the debugging of such a complex system, is in part solved by software (see below) and in part solved by the policy of stepwise construction. As of the end of the reporting period, a sufficient number of components, both hardware and software, are operational so that major subsystems (scanners, displays, mask registers) can be tested and evaluated by applying the as yet incomplete but nevertheless viable system to real biologic material. The results of such applications provide a functional evaluation of existing components and their relationships...on which the addition of successive components may be based. Thus, nothing new is added until performance has been checked against specification. This classic technic for complex system construction has worked for the Grain Counter and seems to be applicable as well to the very much more complex Real Time Picture Processor.

I. Progress - Hardware

1. The Axiomat Microscope has been installed as the central component of the Real Time Picture Processor. As noted in the publications, the Axiomat is particularly suited to this role since it allows for multiple scanners to be applied to the same object. Thus, for the first time it is possible to measure and compensate for some of the positional dependent noise in plumbicon scanners other than by the assumptions on which so-called shade correctors are based. The close cooperation with Zeiss in regard to special modifications and optical interfacing has been particularly gratifying.

2. The Galvanometer-Mirror Precision Scanner has been optically interfaced to the Axiomat and electronically interfaced to the PDP8/e process control computer component of the RTPP. The mirror scanner is capable of operating essentially linearly in either raster or point addressable mode over a field corresponding to 1048 x 1048 points. Thus, the mirror scanner can be made to scan the same object or part of an object which is scanned by the Quantimet 720. Since scanner errors in the case of the mirror scanner and in the case of the 720's plumbicon are entirely different, it is now possible for the first time to evaluate television scanner data objectively and independently. Both the input and output ends of the electronic interfaces were designed and constructed by Morton Schultz.

3. The Quantimet 720 has been interfaced to the PDP8/e process controller. As a result of CAMBION execution of the designs generated by Carman Electronics, the Quantimet including the digitizer, amender and "function" computers, as well as the 1D and standard detector, are now under computer control. Starting with the ability to initiate a scan, the computer **can automatically** command any of the component Quantimet modules to execute one or more measurements (counts, sizings, shape, characterizing, density or area) and the results displayed in registers and/or on the Quantimet monitor.

4. Sonic Digitizer. Schultz and Lemkin have successfully interfaced this peripheral to the PDP8. This allows the user to "point" to an object in the microscopic field, delineate objects of irregular outline for measurement, detection or Quantimet analysis. At the conclusion of the reporting period, the pen position and the result of drawing operations was being presented as superimposed lines over the television/precision digital displays. The effect is essentially that of being able to "scissor" or otherwise "disect" microscopic images in real time, and see immediately the results of such operations.

5. Mask Register. This is the first special purpose memory component of the Real Time Picture Processor and it is responsible for storing property lists of various measured or detected objects as well as displaying some of the results of computer generated transformations of images. The design by Carman Electronics was implemented by CAMBION Corporation and is essential for the testing of the buffer memories and program memories which will be developed during the next two years. At the same time, it has allowed us to perform automatically sequences of measurements on cells which have heretofore been impossible.

6. Control Table. B. Lipkin, Lemkin, Schultz and Hazard have designed and BEIB, DRS has completed a control table which allows the user to control directly all of the components noted above as well as the illumination system described below. This, coupled with an off-the-shelf GT-40, constitutes the user interface for the entire system. Of particular interest is hardware access provided to all present and future computer memory and register components, as well as fully flexible displays.

7. Illumination Subsystem. A broad cooperative project involving close collaboration among the image processing group, Carl Zeiss and Arthur D. Little has resulted in a computer controlled illumination subsystem for the Axiomat. Essentially, via stepping motors, real time random access to any visible wavelength and any relative light intensity, for a wide range of light sources,has become available and functional. Designed by Cook, B. Lipkin and Hazard, any light source may be used by the Axiomat with full computer control of related variables. Sources presently available include: Quartz Iodine, Xenon Arc, Zirconium Arc, and Hydrogen/Deuterium lamps. This flexibility in illumination selection and control is particularly important in view of the practicality demonstrated (in various commercial machines and in the Grain Counter) of wavelength dependent algorithms for all identification and/or analysis.

8. Detectors. In addition to the quantizing detectors, the plumbicon and mirror scanner, two additional "field" type detectors link the Axiomat to the RTPP. One of these, a quartz fiber optic bundle, monitors the time dependent variation in the special illumination system above. The other, a Tektronix J-20 (silicon storage) probe provides a real time visible spectral distribution of the microscopic field. This is particularly important for the anticipated fluorescence microscopic measurements anticipated for the next year. The system will thus be able to handle mythramycin and ethidium bromide fluorescence, despite the insensitivity of plumbicons to the later output. Schultz has had the primary responsibility for interfacing these detectors.

9. Video Multiplexor. The most unusual of the existing hardware components was nearing completion at the end of the reporting period. This device, employing ultra high speed A/D and D/A conversion, allows the simultaneous display of the TV image and one or more processed resultants. This system designed by Schultz and Carman is the visual kernel of the entire RTPP, since it allows, for example, a biologist to see what he has done to an image as soon as the operation is performed. Other uses are the direct comparison, side by side, of mirror and plumbicon scanned images. The main impact of this device will not be apparent until the buffer memories are operational at the end of the next fiscal year, but some effect is already apparent, even in their use in debugging other system components.

II. Progress - Software

The automatic cell analysis system has two major system software aspects. The first of these, the local RTPP monitor/debugging system - DDTG, is now operational. This system allows use of each existing hardware component and its application for obtaining real data on real biologic images. This system is almost entirely the work of Lemkin. DDTG embodies facilities for acquiring images from the plumbicon, mirror scanner, data from the reference channel or the J20 spectrometer, controlling the wave length of illumination and its intensity, the zoom function of the Axiomat and the X-Y motion of the stage, as well as its focus. In respect to the Quantimet 720, DDTG allows the user to initiate plumbicon scans, measure the areas, density, perimeter, projected and Feret diameters on up to several hundred separate objects in a given microscopic field. DDTG also provides service routines for the sonic digitizing tablet, the Quantimet frame and scale controls, the high precision Dicomed display, and various I/O devices of the PDP8/e. Lastly, it is the software interface to the user, via the control table, the various control of which it is constantly monitoring. All mask register operations are under DDTG control.

Provision is made in DDTG for the full range of general picture processor instructions, control of buffer memories, etc., components of the Real Time Picture Processor which will be brought into operation during the next two years. These hardware and software facilities are indicated in the publications by Carman, et al and Lemkin, et al.

The second major component which is being developed on the PDP-10 by Lemkin, Shapiro and Kaiser deals with the facilities described by Shapiro, et al.

This system, which will be resident on the DCRT PDP-10, comprises the higher level relational and logical structures which will allow biologists to construct models and cell analysis systems. Broadly speaking, the artificial intelligence components of an overall automatic system would reside here. CELMOD in fact will be able to initiate actions by the RTPP acting via the RTPP monitor DDTG.

The various components of CELMOD, the parser generator, loader and procedural description language are all under construction in SAIL, the Stanford Artificial Intelligence Language, a facility which we are particularly fortunate to have available on the PDP-10.

The system thus in software, as in hardware, is now capable of doing real analyses on real slides on human command. The immediate process control and special purpose hardware (RTPP) and its software system will also be used interactively to build the practical portions of CELMOD into the theoretic framework of the latter which is now under construction by Lemkin, Shapiro and Kaiser. In this, a particularly noteworthy accomplishment has been Shapiro's development of a parser generator. This program complex when presented with a set of grammatical rules, will generate the appropriate parsing programs for those rules. It forms a vital part of the procedural description language which we are building on the framework of Gary Knott's (DCRT) MLAB system

Keyword Descriptors: Artificial Intelligence
Scanners
Densitometry
Biological Cell Classification
Pattern Recognition
Light Microscopy
Distributed Computing
Cell Kinetics
Bone Marrow
Tissue Cultures

Honors and Awards: None

Publications:

Lemkin, P., Carman, G., Lipkin, L., Shapiro, B., Schultz, M, and Kaiser, P.: A real time picture processor for use in biologic cell identification, I. system design. J. Histochem. Cytochem, 22(7): 725-731, 1974.

Carman, G., Lemkin, P., Lipkin, L., Shapiro, B., Schultz, M. and Kaiser, P.: A real time picture processor for use in biologic cell identification, II. hardware implementation. J. Histochem. Cytochem. 22(7): 732-740, 1974.

Schultz, M., Lipkin, L., Wade, M., Lemkin, P. and Carman, G.: High resolution shading correction. J. Histochem. Cytochem. 22(7): 751-754, 1974.

Shapiro, B., Lemkin, P. and Lipkin, L.: The application of artificial intelligence techniques to biologic cell identification. J. Histochem. Cytochem. 22(7): 741-750, 1974.

Lipkin, L. and Lipkin, B.: Computer Involvement in the Clinical Pathologic Laboratory. In Mullins, L.J. (Ed.): Annual Review of Biophysics and Bioengineering. California, Annual Reviews, Inc., June 1975, Volume 4. In Press.

SUMMARY REPORT

RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1974 through June 30, 1975

The clinical research program described in the Summary Report of last year has been strengthened during the past twelve months by the creation of a medical service in the Radiation Oncology Branch, institution of a medical oncology fellowship program providing broad multidisciplinary training, and an increased emphasis on laboratory investigation. These changes, combined with increasingly greater collaboration with other clinical and basic research units, have served to further broaden the scientific base within the Branch to the extent that activities directly concerned with radiotherapy now constitute a minority fraction of the total effort.

The Ewing's sarcoma program has become a model example of the combined modality treatment concept with accession of a larger number of patients into study than at any other single institution in the U.S.A. Systematic evolution in the manner of integrating radiation therapy and chemotherapy has produced a five-fold increase in the number of long-term, disease-free survivors. This dramatic achievement serves as an eloquent example of the marked potential for improving the therapeutic results in a wide range of malignant diseases, including some of the more common forms of cancer. In this respect, a study has been undertaken on lung cancer during the past year and the preliminary results for undifferentiated carcinomas of the small cell type appear most promising.

A comprehensive review of ten years experience with chronic lymphocytic leukemia was completed during the year. This form of leukemia, newly diagnosed in 5500 patients per annum, has never had the course of disease substantially altered by treatment to date. Carefully documented information on patients with active, symptomatic chronic lymphocytic leukemia using total body irradiation as the principle method of treatment has demonstrated for the first time that, in fact, the natural history of disease can be modified by therapy. One third of patients experienced essentially complete clinical and hematologic remissions and a number of unique observations applied to these remitters, namely return to a normal performance status, recovery from hypoimmunoglobulinemia and absence of infectious complications, and significant prolongation of survival. Achievement of these goals for at least a fraction of patients will provide an enormous stimulus for renewed attention to a disease where the "status quo" has applied for decades.

The long-term observation and data collection on patients treated for Hodgkin's disease between 1965 and 1969 has continued to yield further insight into both the disease itself and the delayed consequences of treatment. The value of this study has been significantly enhanced by carefully maintained observation of every patient treated during the above time interval. The collaborative study of the non-Hodgkin's lymphomas, by virtue of its large-scale and meticulous compilation of data, has contributed to understanding of these protein diseases in a major fashion. Improved appreciation of the biology and kinetics of the malignant lymphomas resulting from this program will almost certainly result in more effective therapeutic management and marked prolongation of survival in the years ahead.

Several highlights deserve comment with respect to the laboratory research program. One staff physicist completed basic research on fast (14Mev) neutrons and the work, submitted in dissertation form, resulted in award of a Doctor of Philosophy from the University of Texas. Newly initiated studies on the kinetic changes in DNA synthesis of murine host and tumor tissues following chemotherapeutic intervention have already produced data currently "in press" and long-term studies on the effects of immunologic manipulation on radiation and chemical carcinogenesis are presently being prepared for publication.

A major disappointment was the failure to implement carefully designed plans for a comprehensive computer system to automate many of the clinical operations of the Branch. A detailed systems design was developed in collaboration with the Biometry Branch, NCI, which would have made historic inroads toward the melding of modern computer technology and patient management in a setting of clinical research. The difficulties encountered were administrative in nature, centering predominantly around contractual mechanisms as presently operational within the Institute.

The staff of the Radiation Oncology Branch has continued to provide extensive advisory and collaborative support to other intramural program areas. As usual, thousands of non-clinical radiation exposures of biological materials and animals were performed, often requiring extensive planning in order to provide high quality support to the respective research efforts. Two-hundred and seventy patients were seen formally in consultation at NIH and approximately 150 of these were treated with radiation therapy. Extensive telephone consultations were provided to over 100 physicians in community practice during the past year. These efforts, while not reflected in the Branch bibliography, are nonetheless viewed as a meritorious contribution.

Project No. Z01 CBD. 00650-20 R
1. Radiation Oncology Branch
2. Radiation Therapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Service Radiation Therapy

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Kent B. Lamoureux, M.D.; Ursula Ruhl, M.D.; Frederick L. Faw, M.S.; Dwight W. Glenn, M.S.; Robert W. Swain, B.S.; Marilyn K. Glover, R.N.; Margaret R. Powell, R.T.

Cooperating Units: None

Man Years:

Total:	8/2
Professional:	3/2
Other:	5/2

Project Description:

Objectives: To provide expert radiotherapy consultation and treatment for Clinical Center patients admitted to services other than the Radiation Branch of the National Cancer Institute.

Methods Employed: During the twelve-month period preceding this report, 270 patients were formally seen in consultation. 291 patients were treated in the department with approximately half of these being service patients, the other half protocol patients of the Radiation Oncology Branch. Advice and management of many additional patients was provided on an emergency or "ad hoc" basis including over 100 logged telephone consultations for advice on treatment or general information. The majority of patients were irradiated with the 6 MeV or the recently installed 12 MeV linear accelerator with electron beam capability following appropriate evaluation and treatment planning as indicated.

Major Findings: Aside from results on research patients as described in project reports from other institutes, the response to radiation therapy has been consistent with that reported from other major centers.

Proposed Course: To continue.

Keyword Descriptors: Radiation therapy

Project No. Z01 CBD. 00650-20 R

Honors and Awards: None

Publications: None

Project No. Z01 CBD. 00684-20 R
1. Radiation Oncology Branch
2. Radiation Therapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Non-Clinical Service Irradiation

Previous Serial Number: Same

Principal Investigator: Robert W. Swain

Other Investigators: Gilbert M. Bowie

Cooperating Units: Laboratory of Physiology, NCI

Man Years:

Total:	3/10
Professional:	1/10
Other:	1/5

Project Description:

Objectives: To provide facilities for the performance of irradiation of biological materials and animals as required for research studies by investigators outside of the Radiation Branch. This includes consultation as to methods and types of irradiation and the preparation of material.

Methods Employed: A dual unit 250 Kvcp x-ray generator is provided and maintained in proper operating condition.

Proposed Course: Because of the age of the equipment and difficulty in obtaining repair parts, new equipment has been ordered in conjunction with the new construction in our section. As a result of a questionnaire circulated to users of this equipment, approximately 60% would use a Cesium Irradiator for their irradiations if it were available. The use of such an irradiator would provide more uniform dosimetry of materials since the only variable would be the exposure time. It is recommended that consideration be given to the purchase of such equipment at a cost of approximately $60,000. Space will be available for this equipment. Maintenance of equipment of this type is minimal and would reduce the wear and tear of the x-ray equipment. The services will continue to be offered.

Keyword Descriptors: Service irradiation

Honors and Awards: None

Publications: None except as indicated by the use of these facilities in publications from other laboratories.

Services Provided Using the 250 KVCP
Double Header Westinghouse Quadrocondex
from April 1, 1974 to April 1, 1975

Organization	No. of Exposures
A-A&R	2
A-LBP	24
C-BGY	4
C-DE	18
C-EXP	4
C-I	208
C-LB	80
C-LBGY	110
C-LCBGY	190
C-LEC	183
C-LMPH	129
C-LPHY	1276
C-LTB	5
C-LCTB	142
C-MET	43
C-MO	10
C-PO	45
C-R	66
C-SURG	30
C-VC	37
C-VLL	28
C-VO	43
CH-BB	4
D-LCI	56
D-LDBA	58
D-LMI	163
D-LOM	58
I-LBV	13
I-LCI	100
I-LI	444
I-LMI	179
I-LVD	9
N-LEN	6
DRS-RS	99
BB-DV	100
*Bionetics	93
*Meloy	50
*Microbiologic	234
	4443

* Exposures for Microbiologic, Bionetics, Meloy and Flow Laboratories were performed on materials for projects under contract to the National Cancer Institute.

Materials irradiated included cells, tissue cultures, blood, mice, rats, hamsters, chickens, and guinea pigs. Exposures ranged from 25 R to 15000 R.

It is estimated that as many as 25% of the exposures made have not been recorded.

Project No. Z01 CBD. 00963-05 R
1. Radiation Oncology Branch
2. Radiation Therapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of Immunologic Manipulation on Radiation Carcinogenesis and Chemical Carcinogenesis and Radiation Response of Tumors

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: John D. Lamberg, M.D., and Walter G. Hardy

Cooperating Units: None

Man Years:

Total:	6/4
Professional:	2/4
Other:	4/4

Project Description:

Objectives: To evaluate the effects of immunologic manipulation on the time of appearance and the incidence of radiation and chemically-induced tumors, and to determine the effect of age at the time of irradiation. Also, to determine the effects of immunologic manipulation on the radiation responses of tumors.

Methods Employed: Total body irradiation or intravenous DMBA in mice with cyclic immunosuppression are the methods utilized to evaluate the alterations and types of tumors and the time of development of radiation and chemically-induced benign and malignant neoplasms. Control groups are expected to reveal differences in spontaneous tumor production under the influence of cyclic immunosuppression. Variations in tumor responsiveness to irradiation by non-specific immunologic suppression and enhancement will be correlated with tumor and tumor bed tissue reactions by histopathologic evaluation.

Major Findings: Immunosuppression delays the onset of lymphomas and accelerates the development of carcinomas and sarcomas in both controls and in mice with induction of tumor by irradiation or DMBA. A comparison of age variations in rapidity of tumor development has been striking in preliminary observation. Preliminary results with non-specific immunologic stimulation with MSF-MB (methanol soluble fraction of Mycobacterium Butyricum) have not provided any significant difference to date though hyperplasia of spleen, thymus, and Peyer's patches has been apparent.

Proposed Course: Complete present carcinogenesis experiments with immunosuppression and immune stimulation utilizing MSFMB. Complete analysis of radiation carcinogenesis studies and prepare for publication.

Keyword Descriptors: Immunologic manipulation; radiation carcinogenesis; chemical carcinogenesis; radiation response

Honors and Awards: None

Publications: None

Project No. Z01 CBD. 00964-04 R
1. Radiation Oncology Branch
2. Radiation Therapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Study of Cancer of the Upper Air Passages and the Evaluation of Combined Treatment Approaches

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Walter G. Hardy; Ralph E. Johnson, M.D.; Janet Rowland, R.N.; and Pat Kelley, R.N.

Cooperating Units: Surgery Branch, Division of Cancer Biology and Diagnosis

Man Years:

Total:	5/4
Professional:	2/4
Other:	3/4

Project Description:

Objectives: To evaluate the usefulness of chemotherapeutic agents singly and in combinations as adjuvants to radiation therapy in patients with malignant squamous cell tumors of the mouth and throat.

Methods Employed: Patients with post-surgical and post-irradiation recurrences and advanced primary inoperable squamous cell carcinomas of the mouth and throat are accepted for study. Drugs currently under study include bleomycin, methotrexate, adriamycin, and vincristine, all of which have shown activity against squamous cell carcinoma. Tumor response to the drugs is evaluated for four to six weeks before initiation of radiation therapy and the drugs are continued during the course of radiation therapy. Great individualization of the radiation is necessary in view of the augmented mucosal reactions from the combined treatment. Currently, randomization is continuing with methotrexate and Bleomycin vs. all four drugs.

Major Findings: Early responses appear significantly better for four drugs in terms of rapidity and completeness of response. This, however, is partially offset by the greater toxicity in terms of skin and mucosal reactions. The long-term comparisons of continued freedom from local recurrence and survival data remain undetermined until more time has elapsed. Preliminary estimates indicate better than 80% complete initial regression of local tumor and probable 30% or better 3-year survival in this very unfavorable group of patients. Studies on sarcoma induction and acceleration of spontaneous tumor induction by immunosuppression associated with I.M. and S.C. administration of bleomycin are complete and being prepared for publication.

Proposed Course: Publish animal studies on bleomycin carcinogenesis. Continue the randomized clinical trial. Results to date, though preliminary, will be presented at a national meeting by request and will subsequently be prepared for publication.

Keyword Descriptors: Radiation therapy; chemotherapy; epidermoid carcinoma; oral cavity; pharynx; bleomycin; methotrexate; adriamycin; and vincristine.

Honors and Awards: None

Publications: None

Project No. Z01 CBD. 00967-04 R
1. Radiation Oncology Branch
2. Radiation Therapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Measurement of "W" for 14 Mev Neutrons

Previous Serial Number: Same

Principal Investigaotr: Dwight W. Glenn, Ph.D.

Other Invesigator: A. P. Wheatley

Cooperating Units: None

Man Years:

Total:	1 1/12
Professional:	1
Other:	1/12

Project Description:

Objectives: To obtain a value of "W" for neutron beams to enable the Bragg-Gray theory to apply to ionization measurements, i.e., relate ionization in a material to the absorbed dose in that material.

Methods: The absorbed dose will be measured in a tissue-equivalent material by determining directly the amount of energy deposited. The amount of energy deposited will be related to the amount of ionization measured in a similar material. (Calorimeter compared to ionization in a tissue-equivalent gas-filled ionization chamber.)

Significance for Cancer Research: Obtaining the value for "W" for neutrons will assist in more accurately determining the comparing tumor doses to patients undergoing radiation treatment with neutron beams. This work would fit Objective 6, Approach 1, of the National Cancer Plan in developing methodologies of radiation.

Proposed Course: The measurements being completed, this project is terminated.

Keyword Description: Measurement of "W" for 14 Mev Neutrons

Honors and Awards: This work, submitted in dissertation form, resulted in the award of the Doctor of Philosophy from the University of Texas to the principal investigator.

Publications: None

Project No. Z01 CBD. 00987-02 R
1. Radiation Oncology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, through June 30, 1975

Project Title: Automatic Data Processing of Clinical Research Information

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Thomas C. Pomeroy, M.D., Janet B. Rowland, R.N.,
Michael Stump, B.S.

Cooperating Units: Biometry Branch, NCI

Man Years:

Total:	5/2
Professional:	5/2
Other:	0

Project Description:

Objectives: Development and implementation of an ADP system for management of clinical research data.

Patient Material: All patients entered into protocol studies are having their clinical records abstracted, coded, and stored on magnetic tape. In the past year, this system was expanded to include all previously treated patients with chronic lymphocytic leukemia and currently there are 680 patients with Hodgkin's disease, malignant lymphoma, Ewing's sarcoma, and CLL with computerized records which are kept updated by continuous editing using an on-line terminal.

Methods Employed: Programs have been developed by the Radiation Oncology Branch staff utilizing the Wilbur system and the DCRT IBM-360 computers. During the year, a system design was undertaken and completed in collaboration with the Biometry Branch, NCI, to expand computer assistance to all major facets of our clinical operation.

Major Findings: The Wilbur system has continued as the mainstay of our patient management and data retrieval operation. The system design for a comprehensive computerized approach in the Branch would have created a model system of uniqueness at the NIH had implementation been possible.

Proposed Course: The ongoing Wilbur program for ADP will be continued and steadily expanded to encompass additional research studies within the Radiation Oncology Branch as well as to support activities in other clinical units. The development of the comprehensive computerized system, which absorbed fully one man-year of professional time, was halted because of the relative lack of administrative support for our efforts to obtain the requisite technical assistance by contract.

Keyword Descriptors: Automatic Data Processing

Honors and Awards: None

Publications: None directly

Project No. Z01 CBD. 00988-10 R
1. Radiation Oncology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Study of Chronic Lymphocytic Leukemia

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigator: Ursula Rühl, M.D.

Cooperating Units: None

Man Years:

Total:	1/1
Professional:	1/1
Other:	0

Project Description:

Objectives: This study is directed toward understanding the epidemiology, natural history, and management of chronic lymphocytic leukemia.

Patient Material: Eighty consecutive patients with previously untreated chronic lymphocytic leukemia have had their clinical records reviewed in detail.

Methods Employed: Information related to epidemiology, potential carcinogenic exposure, family history, natural history of disease, immunologic parameters, treatment and response, intercurrent medical problems, and survival have been encoded, computerized, and retrieved for analysis.

Major Findings: Evaluation of the extensive data bank now available in a retrievable form is currently underway. The preliminary observations clearly indicate for the first time, therapeutic responses have been achieved which have materially altered (improved) the natural history of disease in patients with chronic lymphocytic leukemia.

Proposed Course: The analysis of data is being completed and material is currently being prepared for publication. In light of the extremely encouraging results, a number of additional patients have been accessioned into study and this will continue during the coming year.

Keyword Descriptors: Chronic Lymphocytic Leukemia

Honors and Awards: None

Publications: None

Project No. Z01 CBD. 00989-10 R
1. Radiation Oncology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Study of Hodgkin's Disease

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Clinical Associates

Cooperating Units: Medicine Branch, Surgery Branch, and Laboratory of Pathology, NCI
Diagnostic Radiology and Nuclear Medicine, CC, NIH

Man Years:

Total:	1/1
Professional:	1/1
Other:	0

Project Description:

Objectives: To understand the natural history of Hodgkin's disease, assess the reliability of diagnostic methods used to guide treatment, examine the effectiveness of radiotherapy and chemotherapy used singly and in combination, and to document the long-term results of treatment and the effects of both treatment and the underlying disease process on the quality of survival.

Patient Material: Two hundred patients with previously untreated Hodgkin's disease were entered into prospective protocol studies beginning in 1965. Accession of patients to the study has now been terminated.

Methods Employed: A collaborative effort involving many cooperating units was directed toward each patient to systematically evaluate the biologic characteristics of Hodgkin's disease, the methods used for diagnostic study, the accuracy of various staging procedures, and comparative clinical trials were undertaken to test the efficacy of various therapeutic techniques.

Major Findings: These have been detailed in the publications listed below.

Proposed Course: Continued observation of patients under study.

Keyword Descriptors: Hodgkin's Disease

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Brereton, H.D., and Johnson, R.E.: Calcification in mediastinal lymph nodes after radiotherapy of Hodgkin's disease. Radiology 112: 705-707, 1974.

Gutensohn, N., Li, F., Johnson, R.E., and Cole, P.: Hodgkin's disease: Tonsillectomy and family size. New Engl. J. Med. 292: 22-26, 1975.

Johnson, R.E., Kun, L.E., Belladonna, J.A., Johnson, S.K., Brereton, H.D., and Cohen, G.A.: Hematologic recovery and deterioration after "successful" radiotherapy for Hodgkin's disease. Ann. Inter. Med. 80: 213-216, 1974.

Johnson, R.E., Thomas, L.B., Johnson, S.K., and Johnston, G.S.: Correlation between abnormal baseline liver tests and long-term clinical course in Hodgkin's disease. Cancer 33: 1123-1126, 1974.

Kun, L.E., and Johnson, R.E.: Hematologic and immunologic status in Hodgkin's disease five years after radical radiotherapy. Cancer (In Press).

ARTICLES IN A BOOK:

Johnson, R.E.: Radiotherapy of Hodgkin's Disease and the Malignant Lymphomas. In Musshoff, K. (Ed.): Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. Berlin, Germany, Springer-Verlag, 1974, Vol. 46, pp. 176-181.

Johnson, R.E.: The Role of "Selective" Staging Laparotomy in Hodgkin's Disease and the Malignant Lymphomas. In Varco and Delaney (Eds.): Controversy in Surgery (In Press).

Canellos, G.P., DeVita, V.T., Arseneau, J.C., and Johnson, R.E.: Carcinogenesis by Cancer Chemotherapeutic Agents: Second Malignancies Complicating Hodgkin's Disease in Remission. In Mathe, G. (Ed.): Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. New York, New York, Springer-Verlag, 1974, pp. 108-112.

Johnson, R.E., and Kun, L.E.: A Discussion of the Influence of Histology on Treatment Selection and Prognosis for Hodgkin's Disease and Malignant Lymphomas. In Vaeth, J.M. (Ed.): Frontiers of Radiation Therapy. New York, New York, S. Karger Publishers, Vol. 8, 1974, pp. 217-220.

Project No. Z01 CBD. 00990-10 R
1. Radiation Oncology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Study of Non-Hodgkin's Lymphomas (Malignant Lymphoma)

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Staff of the Medicine Branch, Laboratory of Pathology, and Radiation Oncology Branch, NCI

Cooperating Units: Medicine Branch and Laboratory of Pathology, NCI

Man Years:

Total:	10/2
Professional:	8/2
Other:	2/2

Project Description:

Objectives: To increase our understanding of the natural history of the malignant lymphomas, to assess the methods of diagnostic evaluation including histopathology, and to evaluate therapeutic approaches via carefully designed and prospective clinical trials.

Patient Material: Approximately 300 patients have been accessioned into this collaborative study.

Methods Employed: Extensive diagnostic and immunologic profiles are compiled prior to initiation of therapy and continued serially during the treatment and post-treatment phase. Various new treatment techniques are being researched in controlled clinical trials.

Major Findings: Significant results have been obtained with respect to methods of diagnostic evaluation, the natural history of these diseases, and understanding of the general therapeutic approaches which must be undertaken for effective management. Evaluation of various chemotherapeutic and radiotherapeutic techniques continues and total body irradiation has been documented to have marked effectiveness for remission induction in selected histologic subtypes, even in the presence of anatomically disseminated involvement.

Proposed Course: Accession of patients into study is continuing with particular emphasis on the development of therapeutic methods yielding more consistent results with remission induction and consolidating the remissions so as to achieve a higher incidence of long-term, disease-free survivors.

Keyword Descriptors: Malignant Lymphomas

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Johnson, R.E.: The management of generalized malignant lymphoma with "systemic" radiotherapy. Brit. J. Cancer(In Press).

Johnson, R.E.: Total body irradiation (TBI) as primary therapy for advanced lymphosarcoma. Cancer 35: 242-246, 1975.

Brereton, H.D., Anderson, R., Johnson, R.E., and Schein, P.: Hyperphosphatemia and hypocalcemia following chemotherapy of Burkitt's lymphoma. Arch. Int. Med. 135: 307-311,1975.

Canellos, G.P., DeVita, V.T., Young, R.C., Chabner, B.A., Schein, P.S., and Johnson, R.E.: Therapy of advanced lymphocytic lymphoma: A preliminary report of a randomized trial between combination chemotherapy (CVP) and intensive radiotherapy. Brit. J. Cancer(In Press).

Chabner, B.A., Johnson, R.E., Canellos, G.P., Young, R.C., Schein, P.S., and DeVita, V.T.: Diagnosis of liver involvement in non-Hodgkin's lymphoma. Clin. Oncol.(In Press).

ARTICLES PUBLISHED IN A BOOK

Johnson, R.E.: Radiotherapy of Hodgkin's Disease and the Malignant Lymphomas. In Musshoff K. (Ed.): Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. Berlin, Germany, 1974, Vol. 46, pp. 176-181.

Johnson, R.E.: Radiotherapy in the Management of Stage III-IV Non-Hodgkin's Lymphomas. In Bucalossi, P. (Ed.): Ilinfomi Maligni. Milano, Italy, Casa Editrice Ambrosiana, 1974,

Johnson, R.E.: The Role of "Selective" Staging Laparotomy in Hodgkin's Disease and the Malignant Lymphomas. In Varco and Delaney (Eds.): Controversy in Surgery.(In Press).

Johnson, R.E., Chretien, P.B., and O'Conor, G.T.: Influence of Extensive Biopsy Sampling at Laparotomy on Planning of Radiotherapy in Non-Hodgkin's Lymphomas. In Musshoff, K. (Ed.): Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. Berlin, Germany, Springer-Verlag, 1974, Vol. 46, pp. 128-131.

Project No. Z01 CBD. 00992-01 R

1. Radiation Oncology Branch
2. Radiation Medicine
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Kinetic Studies of DNA in Host Tissues and Tumor Tissues following Chemotherapy

Previous Serial Number: None

Principal Investigator: Harmar D. Brereton, M.D.

Other Investigators: Thomas Bryant, B.A., Robert C. Young, M.D.

Cooperating Units: None

Man Years:

Total:	6/4
Professional:	2/4
Others:	4/4

Project Description:

Objectives: To evaluate the kinetic changes induced in normal tissues and tumorous tissues following chemotherapeutic intervention and to design optimal drug schedules with this information.

Methods: BDF_1 mice bearing B_{16} melanomas were treated with methyl CCNU. Alterations in DNA synthesis in host tissues and tumor tissues were measured.

Major Findings: An optimal dose schedule for methyl CCNU in this system would have been approximately every six days; however, the rapid resistance is permanent, lasting through many generations. The methods employed may provide a ready means of evaluating drug efficacy in solid tumors in other animal systems.

Proposed Course: Continue this work in other solid tumors with other chemotherapeutic agents and radiation therapy.

Keyword Descriptors: Tumor kinetics, B_{16} melanoma, Methyl CCNU.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Brereton, H.D., Bryant, T., and Young, R.C.: "Inhibitions and recovery of DNA synthesis in host tissues and sensitive and resistant B_{16} melanoma after methyl CCNU - A predictor of clinical efficacy", Cancer Research (In press).

Project No. Z01 CBD. 00994-04 R
1. Radiation Oncology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Treatment of Lung Cancer (Undifferentiated Small Cell Type)

Previous Serial Number: NCI-975(c)

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Harmar D. Brereton, M.D., Lawrence L. Michaelis, M.D.

Cooperating Units: National Heart-Lung Institute

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives: The nearly consistent failure of surgery and/or radiotherapy to cure patients with undifferentiated (small cell type) carcinoma of the lung because of disseminated disease is a universal experience. Conversely, a pilot study attempting to achieve disease control using intensive combination chemotherapy in our Branch met with failure because of the inability to eradicate bulky tumor within the confines of the thoracic cage. The obvious conceptual approach is now being utilized, namely the integration of a modality with proven local effectiveness (radiotherapy) with a systemic modality using chemotherapeutic agents of documented activity.

Patient Material: This study has only recently been initiated and a small number of patients have been accessioned to date.

Methods Employed: In view of the predictable natural history of these carcinomas, exhaustive diagnostic evaluation has been moderately curtailed to facilitate the rapid institution of therapy. The pretreatment workup is primarily confined to evaluation of factors which may influence treatment toxicity, e.g., bone marrow, hepatic, and renal function, as well as conditions which may complicate the early management (pulmonary function and possible metastases to the central nervous system or bone). An integrated treatment approach analogous to that developed for Ewing's sarcoma is being employed, namely concurrent radiotherapy to the primary tumor and mediastinal lymphatics, prophylactic irradiation of the brain, intrathecal methotrexate (with systemic citrovorum), and systemic combination chemotherapy. The latter is continued for several months following completion of radiation therapy.

Major Findings: The study has not been in progress for a sufficient period of time for evaluation of the results.

Proposed Course: Continued accession of patients.

Keyword Descriptors: Lung Cancer

Honors and Awards: None

Publications: None

Project No. Z01 CBD. 06502-10 R
1. Radiation Oncology Branch
2. Radiation Therapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Study of Ewing's Sarcoma as a Model System in Experimental Therapeutics for Adjuvant Chemotherapy and Immunotherapy

Previous Serial Number: Same

Principal Investigators: Thomas C. Pomeroy, M.D.

Other Investigators: Ralph E. Johnson, M.D.; Robert K. Oldham, M.D.; Harmar D. Brereton, M.D.; Adelbert Evans, Ph.D.; Janet Rowland, R.N.; and Pat Kelley, R.N.

Cooperating Units: Laboratory of Cell Biology, Division of Cancer Biology and Diagnosis; and the Armed Forces Radiobiological Research Institute.

Man Years:

Total:	5/4
Professional:	4/4
Other:	1/4

Project Description:

Objectives: To determine the value of adjunctive chemotherapy and immunotherapy in the management of Ewing's sarcoma. The evaluation of glycoprotein profiles as an indicator of total tumor burden will be studied in conjunction with the laboratory at the Armed Forces Radiobiological Research Institute. Evaluation of immune competence and the potential for nonspecific and specific immunotherapy will be carried out with studies available through the Laboratory of Cell Biology. Analysis of specificity of LDH as a prognostic indicator will be expanded to evaluation of LDH isoenzymes. Patients with and without known metastatic disease will be admitted to this study.

Methods Employed: Adjuvant therapy with active chemotherapeutic agents is combined with primary radiation therapy and prophylactic irradiation of the central nervous system. Evaluation of immune competence is being studied by the use of microcytotoxicity assays of the patients' lymphocytes against Ewing's cell lines and other cell lines. T-rosetting, lymphocyte stimulation and macrophage inhibition factors are also included. In the future, serum that is now being stored will be evaluated for the presence of blocking antibodies. Nonspecific and specific immunotherapy has been initiated in patients with metastatic disease and patients at high risk (>50%) of relapse. The determination of glycoprotein profiles will be obtained serially on all patients initially, during treatment, and followup after treatment.

Major Findings: Five-year survival rates in patients presenting without any evidence of clinical metastasis have exceeded fifty percent as contrasted with the expected survival of approximately ten percent ten years ago. There has been a similar improvement in disease control in patients presenting with metastasis since the addition of adriamycin into the protocol which was initiated in November, 1971. Current experience with the adriamycin protocol in the prophylactic treatment indicates a potential for greater than seventy percent five-year survival. Preliminary evidence indicates that the immune competence determinations may provide prognostic information as well as provide some method of selection for addition of non-specific and/or specific immunotherapy. Glycoprotein profiles have shown some correlation with continued presence of active tumor and in some instances have predicted relapse a few months before the relapse was clinically evident. Initial LDH levels have accurately predicted relapse and progression of disease.

Proposed Course: Continue patient acquisition for further evaluation of treatment methods and to provide serial laboratory evaluation of immune competence and further evaluation of glycoprotein profiles and LDH as a measure of total tumor load and/or tumor activity. The utilization of non-specific and specific immunotherapy has been initiated for patients with progressive disease and at high risk for relapse.

Keyword Descriptors: Ewing's sarcoma; adjuvant chemotherapy; immunotherapy.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Brereton, H.D., Simon, R., Pomeroy, T.C.: Pretreatment serum lactate dehydrogenase predicting metastatic spread in Ewing's sarcoma. Ann. Intern. Med. (in press).

Cattell, H.S., and Pomeroy, T.C.: Iatrogenic fractures of biopsy sites in long bones. J. of Bone and Joint Surg. (accepted for publication).

Johnson, R.E., and Pomeroy, T.C.: Evaluation of therapeutic results in Ewing's sarcoma. Am. J. Roentgenol. Radium Ther. Nucl. Med. (in press).

Merrill, J., Greco, F.A., Zimbler, H., Brereton, H.D., Lamberg, J.D., and Pomeroy, T.C.: Adriamycin and radiation: synergistic cardiotoxicity. Ann. Intern. Med. 82:122, Jan. 1975.

Pomeroy, T.C., and Johnson, R.E.: Combined modality therapy of Ewing's sarcoma. Cancer 35:36-47, 1975.

Pomeroy, T.C., and Johnson, R.E.: Prognostic factors for survival in Ewing's sarcoma. Am. J. Roentgenol. Radium Ther. Nucl. Med. (in press).

Zimbler, H., Robertson, G.L., Bartter, F.C., Delea, C.S., and Pomeroy, T.C.: Ewing's sarcoma as a cause of SIADH. J. Clin. Endocrinol. Metab. (accepted for publication).

ARTICLES IN A BOOK:

Johnson, R.E. and Pomeroy, T.C.: Integrated therapy for Ewing's sarcoma. Progress in Clinical Cancer, 1974 (in press).

Pomeroy, T.C. and Johnson, R.E.: Integrated therapy of Ewing's sarcoma. Frontiers of Radiation Therapy and Oncology (in press).

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1974 through June 30, 1975

This has been a year of transition for the Surgery Branch. On July 1, 1974, Dr. Steven A Rosenberg became Chief of the Surgery Branch, replacing Dr. Alfred Ketcham, who had held that position for the past 17 years. With Dr. Rosenberg came a change in philosophy and emphasis within the Surgery Branch. There have been significant changes in senior staff personnel, organization of the clinical and teaching services, and changes in research directions.

Over the past decade the Surgery Branch at the National Cancer Institute developed an international reputation for excellence in the radical surgical approach to cancer treatment. The Surgery Branch has been a major center for the development and application of new radical surgical operations. The development and application of the craniofacial exenteration, major pelvic exenterative surgery, and major head and neck resective and reconstructive surgery has been a hallmark of the Surgery Branch's activities in the past.

In accordance with Dr. Rosenberg's emphasis on combined modality treatment and the strong involvement of surgical oncologists in the postoperative adjunctive modalities of immunotherapy and chemotherapy, a shift in emphasis towards these directions is in progress. The future development of the Surgery Branch will be guided by the principle that the future of surgical oncology lies not in the direction of bigger and wider cancer resections but rather with a strong commitment to and involvement in adjunctive treatment. The realization that two-thirds of patients already have disseminated disease at the time they first consult a surgeon requires that the surgeon gain expertise in the control of disseminated disease as well as the local control of cancer. Towards this end, the clinical and laboratory efforts of the Surgery Branch will be devoted to the development of innovative immunologic and other approaches to the treatment of malignant disease to be used as surgical adjuvants. The Surgery Branch will also place emphasis on its involvement in the administration of these adjuncts in many of its protocols.

In keeping with this philosophy the Surgery Branch this year submitted several protocols to the NIH Clinical Research Committee involving the combined modality treatment of cancer. One protocol, for example, on the treatment of primary malignant melanoma involves the joint participation of the Surgery, the Immunology, Pathology, and Biology Branches of the NCI. Another protocol submitted near the end of this year is devoted to the combined modality approach to the treatment of soft tissue sarcomas. In this protocol the Surgery Branch has enlisted the cooperation and close collaboration of the Radiation Oncology Branch and the Chemotherapy Branch. This protocol will be asking questions relating to the best surgical treatment for these cancers and the role of postoperative adjuvant radiotherapy, chemotherapy, and immunotherapy. The Surgery Branch is involved in an extensive review of its clinical activities in an attempt to develop innovative combined modality treatment protocols for a variety of diseases. Towards this end the major emphasis that the Surgery Branch has had in the past on major radical exenterative surgery is being de-emphasized.

There are many new people who have joined the Surgery Branch in the last year or who will be joining in the near future. Dr. Alan Baker joined the senior staff of the Surgery Branch July 1, 1974. Dr. Baker brings with him an expertise in clinical surgery as well as extensive experience and interest in tumor immunology. Dr. Baker completed his medical school and residency training at Harvard and has rapidly become a major contributing member of the Surgery Branch.

On April 28, 1975, Dr. Murray Brennan joined the Surgery Branch as head of the newly created Surgical Metabolism Section. Dr. Brennan is nationally known in the field of surgical metabolism and hyperalimentation and will be developing both clinical and laboratory programs in this area oriented towards elucidating nutritional factors important in tumor-host relationships and their possible role in the management of patients with cancer. Dr. Brennan will be organizing a clinical service in nutrition and intravenous hyperalimentation that will be available to serve the entire clinical needs of the National Institutes of Health.

Shortly after the summer a major reorganization of the Surgery Branch clinics was undertaken. All patients were assigned to individual clinical associates or senior staff and continue to see that same physician during his entire tenure at the NIH. All patients are now seen in the Surgery Branch clinics by specific appointment with "their" physician and continuity of care becomes a primary consideration.

The addition of Ms. Claudia Seipp, a former head nurse both on the surgical ward and in the surgical clinic, as a full time member of the Surgery Branch has been an immense help in the reorganization of our clinical structure. Ms. Seipp has had extensive experience in the organization of clinical care and studies and is heavily involved in the management and organization of our new clinical protocols.

At the beginning of September 1974, the surgical training program was modified. Surgical Grand Rounds were instituted to enable invited speakers, both from within and outside of the NIH, to give formal presentations of their work. This has provided a forum to keep the Surgery Branch abreast of recent developments in surgery and related fields. A conference is also held weekly immediately preceding the Surgical Grand Rounds to discuss the preoperative cases for the following week. This enables the entire staff of the Surgery Branch to provide clinical input into the management of its patients.

Chiefs Rounds was organized on Tuesday afternoons. During these rounds, Dr. Rosenberg meets with the senior staff and residents on the ward service to discuss individual patient management problems in more detail than is possible at other sessions. A daily meeting between the Surgery Branch and the Radiology Department to review patient x-rays has also been instituted.

Because of the changes in emphasis listed above, much of the research in the Surgery Branch is also undergoing a change in direction. Major emphasis is being placed on the study of the basic immunobiology of cancer and in detailed studies of the immune response of the host to malignancy. The goal of these studies is the development of new methods of patient immune monitoring as well as the development of new immunotherapies. The Surgery Branch is developing the capability to perform a wide variety of measurements of the immune competence of cancer patients. An extensive program has begun on the cryopreservation of lymphocytes and tumor cells that will enable retrospective studies of the immune response of patients. The Surgery Branch has a serum bank that contains over 15,000 samples from over 4,000 patients. A lymphocyte bank is currently being organized to complement these studies.

Surgical procedures and consultations performed by the Surgery Branch from April 1, 1974 to March 31, 1975 are presented in Tables I-V.

Serial No. Z01-CBD-03800-05 SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Surgical Consultations and Collaborative Research Involving Surgical Services at the National Institutes of Health

Previous Serial Number: Same

Principal Investigators: Steven A. Rosenberg, M.D., Ph.D. and entire staff of Surgery Branch, NCI

Other Investigators: G.D. Aurbach, M.D., F.C. Bartter, M.D., V.T. DeVita, M.D., J.L. Doppman, M.D., A. DuBois, M.D., J.R. Gill, M.D., R.E. Johnson, M.D., D.M. McCarthy, M.D., R.D. Powell, M.D., J. Robbins, M.D., R.J. Sherins, M.D., L.B. Thomas, M.D., R.C. Young, M.D., and J.L. Ziegler, M.D.

Cooperating Units: Metabolic Diseases Branch, NIAMDD; Division of Intramural Research, OD, NHLI; Division of Cancer Treatment, NCI; Diagnostic Radiology Department, CC; Digestive Diseases Branch, NIAMDD; Division of Intramural Research, NHLI; Radiation Oncology Branch, NCI; Division of Heart and Vascular Diseases, NHLI; Laboratory of Pathology, NCI; Clinical Endocrinology Branch, NIAMDD; Reproduction Research Branch, NICHHD; Medicine Branch, NCI; Pediatric Oncology Branch, NCI

Man Years:

Total:	6
Professional:	4
Other:	2

Introduction

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

There is a particular emphasis in our collaborative studies on endocrine diseases. During the past 12 months the Surgery Branch performed operations on 27 patients with parathyroid disease, seven patients with thyroid disease, and six patients with adrenal tumors. We performed a total of 204 diagnostic biopsies, most of which were in our capacity as a consultant for other services. A complete listing of our surgical procedures is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

The Surgery Branch is heavily involved in providing outpatient ENT consultations through its staff of consulting otolaryngologists. The extent of this work is presented in Table III.

A list of consultations received from other NCI branches as well as other NIH institutes is shown in Table IV.

The operative case load in the NIH surgical suites by referring institute is shown in Table V.

Project Description:

Part I.	Hyperparathyroidism
Part II.	Role of Splenectomy in the Management of Hematologic Abnormalities
Part III.	Studies of Gastric Function in Postoperative Patients

Keyword Descriptors: Surgery, hyperparathyroidism, splenectomy, gastric function

Project Description:

Part I. Hyperparathyroidism

Objectives:

The Clinical Endocrinology Branch of the National Heart and Lung Institute and the Section of Mineral Metabolism of the Metabolic Diseases Branch, NIAMDD, and the Diagnostic Radiology Branch, Clinical Center, have programs underway to determine clinical, laboratory and radiographic criteria which justify surgical exploration of the neck for the treatment of parathyroid disease.

Methods Employed:

Routine surgical techniques and anesthetic procedures are utilized when operating on the patients. Extensive clinical evaluation and laboratory determination of derangements in calcium and phosphorous metabolism were performed. Additionally, selective inferior thyroid arteriography and selective neck vein catheterization for localized sampling of parathormone in the area of suspect disease are performed. Immunoassays are utilized to determine the level of PTH in various samples and hopefully as an aid to localize and predict the type of disease.

Major Findings:

From April 1, 1974 to April 1, 1975, 27 patients were subjected to clinical and surgical evaluation. Thirteen of these patients have been operated on previously at other institutions. Venographic studies of these patients is probably of most value in the group of patients requiring re-exploration. Eleven out of 13 patients have an accurate preoperative prediction of localization of their disease, by utilizing venographic sampling and PTH assay. Three of the 13 patients operated on for continued hyperparathyroidism after previous surgical resection continued to have hypercalcemia after the second operation. However, the remaining 10 patients were cured of their hyperparathyroidism with minimal morbidity and no mortality.

These studies represent an ongoing interest in the surgical management of hyperparathyroidism. In the period between 1955 and 1974, the Surgery Branch evaluated and operated upon 150 patients with a preoperative diagnosis of hyperparathyroidism. During this year this experience was reviewed for publication and this extensive experience has contributed to an understanding of the management of this clinical problem. Some of the Surgery Branch experience is summarized in Tables 6 and 7.

Table 6

OPERATIVE PROCEDURE

	PRIMARY OPERATION	REOPERATION
EXCISION OF ADENOMA	15	10
EXCISION OF ADENOMA AND BIOPSY OF NORMAL GLANDS	50	4
EXCISION OF 3-1/2 GLANDS	36	0
EXCISION OF LESS THAN 3-1/2 GLANDS	25	8
BIOPSY OF ALL GLANDS ONLY	10	0
NO GLANDS FOUND	0	2

Table 7

OPERATIVE RESULTS

	PRIMARY OPERATION	REOPERATION
NORMOCALCEMIC POST-OP	93	12
TEMPORARY HYPOCALCEMIA	28	1
PERMANENTLY HYPOCALCEMIC	2	7
CONTINUED HYPERPARATHYROIDISM	3	3
CORD PALSY	0	5
OPERATIVE DEATH	0	1

Significance to Cancer Research and Program of the Institute:

Early diagnosis and removal of hyperactive parathyroid tissue is an important step toward the prevention of renal disease secondary to hyperparathyroidism. Selective venous catheterization and PTH immunoassay are proving to be of great assistance in localizing and predicting disease preoperatively. Detailed metabolic analysis of patients pre-, during, and postsurgery should prove of value in predicting success or failure of surgical treatment before the expected serum calcium drop.

NCP Objectives and Approach: 04, A1,2; 06, A1

Proposed Course:

This project will continue with further evaluation of diagnostic techniques such as venous catheterization studies in the management of patients with primary hyperparathyroidism, particularly those patients who have had previous unsuccessful surgery. Studies on the preservation and autotransplantation of parathyroid tissue in selected patients are under consideration.

Project Description:

Part II. Role of Splenectomy in the Management of Hematologic Abnormalities

Objectives:

To evaluate the usefulness of splenectomy in the management of various hematologic abnormalities such as hereditary spherecytosis and anemia of Felty's Syndrome and in chronic myelogenous leukemia.

Methods Employed:

A standard midline surgical approach is utilized, based upon the Surgery Branch's extensive experience in splenectomy for the staging of Hodgkins Disease. Generally, exploration is coupled with a liver biopsy and significant lymph node biopsy in addition to the splenectomy. Thirteen splenectomies were done between April 1974 and April 1975 for chronic myelogenous leukemia, Felty's Syndrome, and spherocytosis and in three patients with lymphoma.

Major Findings:

In the management of chronic myelogenous leukemia, splenectomy appeared to have a role in patients with drug-induced thrombocytopenia. Following splenectomy there was usually a rapid rise in platelet count and cessation of bleeding tendencies.

In Felty's Syndrome, there appears in some patients to be a mild anemia which responds to splenectomy, thus reducing the need for periodic blood transfusions.

In spherocytosis the hemolytic anemia is eliminated resulting in prolonged red cell survival and less dependency on transfusion.

Significance to Cancer Research and Program of the Institute:

This study is aiding in delineation of a role for splenectomy in the management of various hematological abnormalities including chronic myelogenous leukemia, Felty's Syndrome, and hereditary spherocytosis. In these three entities hematologic gains occur after splenectomy.

Information gained as a result of this therapeutic intervention has improved clinical management and treatment of these diseases.

NCP Objectives and Approach: 04, A1,2; 06, A1

Proposed Course:

The project will continue with further exploration of the beneficial effects of splenectomy on the course of patients with chronic myelogenous leukemia. As patients with other hematological abnormalities are studied, the effect of splenectomy will be evaluated along with immunological alterations precipitated by splenectomy.

Project Description:

Part III. Studies of Gastric Function in Postoperative Patients

Objectives:

To determine if the changes in postoperative gastric function can be related to sympathetic nervous system activity.

Methods Employed:

The rate of gastric emptying and the rate of secretion of acid and water are measured simultaneously using dye dilution procedures. A nasogastric tube is passed into the stomach of a fasting subject. The gastric juice is aspirated and the stomach washed with 50 ml distilled water. Using the nasogastric tube, up to 750 ml of a liquid test meal composed of distilled water and phenol red (40 mg/L) will be administered into the stomach. At ten minute intervals for the subsequent 50 minutes, 10 ml of gastric contents are aspirated and 20 ml of a solution identical in composition to the test meal except that the phenol red concentration is 280 mg/L is infused into the stomach. Mixing of gastric contents is achieved by withdrawing and reinserting syringefuls of gastric contents for one minute, and then a second 10 ml sample is aspirated. At the end of the procedure, the stomach is completely emptied and then washed out with 200 ml distilled water.

The concentration of phenol red, H+, Na+, K+ and Cl- ions are determined in all samples. From these data we calculate the intragastric volume and acid and electrolyte content at each ten minute time point, the amount of emptying in each ten minute interval, and, by difference, infer the rates of secretion of acid, volume, and electrolyte.

Major Findings:

The rates of gastric emptying and secretion have been evaluated in six surgical patients undergoing exploratory laparotomy. A technique that combines dye dilution and rapid sampling has been applied preoperatively and 24 hours after surgery. An additional study was performed on the third postoperative day in two patients. Rates of emptying and secretion were dramatically changed on the first postoperative day, as compared to the preoperative values: the fractional rate of emptying was markedly decreased, the rate of water secretion was unchanged or decreased while the rate of hydrogen ion secretion was decreased. These changes resulted in a decrease in the hydrogen ion concentration of the secreted fluid (58 ± 6 mEq/L preoperatively to 37 ± 7). By the third postoperative day gastric emptying was still decreased (0.05 ± 0.02 per minute) and water secretion was normal; however, hydrogen ion concentration in the secreted juice exceeded by 50% that observed during the preoperative period (78 ± 9 mEq/L).

Significance to Cancer Research and Program of the Institute:

These results show that, following abdominal surgery, not only are gastric secretion and emptying impaired but the pattern of changes is such that emptying decreases to a greater extent than does secretion. This combination tends to produce gastric distention, which might be one etiologic factor responsible for the diminished gastric blood flow known to occur during the early postoperative period. Although gastric secretion of acid is initially reduced, by the third postoperative day it exceeds by 50% that observed preoperatively. This change, coupled with the persistent reduction in gastric emptying, results in a prolonged exposure of the gastric mucosa to a more acidic solution which, in turn, may be important in the pathogenesis of postoperative gastric hemorrhage.

NCP Objectives and Approach: 04, A1,2; 06, A1

Proposed Course:

Four additional patients should be studied pre- and postoperatively to confirm these findings. The data collected in this way will be used to construct a mathematical model of the gastric secretions and emptying using the SAAM computer program. In the dog, such a model permitted an analysis of the surgically induced changes of the gastric functions. It also stressed the powerful prophylactic effect of sympathetic blockade. This procedure does not block the adrenergic receptors and does not prevent the vaso-motor effects of circulating catecholamines. Its use in postoperative patients should therefore be considered in a double blind controlled study.

Publications:

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Serial No. Z01-CBD-03801-05-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Studies in Cancer Surgery

Previous Serial Number: Same

Principal Investigator: S.A. Rosenberg, M.D., Ph.D., and the entire staff of the Surgery Branch, NCI

Other Investigators: L.B. Thomas, M.D., R. Powell, M.D., J. Costa, M.D., A.S. Rabson, M.D.; W. Terry, M.D.; R.C. Young, M.D., V.T. DeVita, Jr., M.D., B.A. Chabner, M.D., G.P. Canellos, M.D., J.L. Ziegler, M.D.; D.C. Tormey, M.D., M. Lippman, M.D.; R.E. Johnson, M.D., T.C. Pomeroy, M.D., H.D. Brereton, M.D.; J.L. Doppman, M.D.

Cooperating Units: Laboratory of Pathology, NCI; Immunology Branch, NCI; Medicine Branch, NCI; Medical Breast Cancer Section, Medicine Branch, NCI; Radiation Oncology Branch, NCI; Diagnostic Radiology Department, CC

Man Years:

Total:	9
Professional:	6
Other:	3

I. Project Description: Surgical Treatment of Malignant Melanoma

Part a. Natural History

Part b. Immunotherapy and Chemotherapy as Post-Surgical Adjuvants in the Treatment of Stage II Disease

Part c. Evaluation of Staging Laparotomy

Part d. Studies on Intralesional Treatment

Keyword Descriptors

II. Project Description: Surgical Treatment of Sarcomas

Keyword Descriptors

III. Project Description: Surgical Treatment of Head and Neck Cancer

Part a. Innovative Surgical Techniques for the Resection of Head and Neck Malignancies and Post-Resection Reconstruction

Part b. The Use of Methotrexate as a Preoperative Adjuvant in Patients with Head and Neck Cancer

Keyword Descriptors

IV. Project Description: Surgical Treatment of Carcinoma of the Cervix

Keyword Descriptors

V. Project Description: Surgical Studies in Breast Cancer

Part a. Detection of Early Breast Cancer

Part b. Treatment of Primary Breast Cancer

Part c. Treatment of Metastatic Breast Cancer

Part d. Treatment of Male Breast Cancer

Keyword Descriptors

VI. Project Description: Genitourinary Aspects of Cancer Management

Part a. Study of Urinary Diversion in Pelvic Malignancy

Part b. Management of Massive Bladder Hemorrhage Secondary to Cyclophosphamide or Radiation Therapy in Cancer Patients

Part c. Tumor Markers in Patients with Testicular Germ Cell Tumors

Keyword Descriptors

VII. Project Description; Endoscopic Studies

Part a. Laparoscopy as a Staging Modality for Patients with Malignant Disease

Part b. Colonoscopy

Part c. Mediastinoscopy in Patients with Carcinoma of the Breast

Keyword Descriptors

VIII. Project Description: Microbiologic Studies in Cancer Patients

Part a. Changes in Bacterial Colonization in the Surgical Cancer Patient

Part b. Antibiotics in Tissue Fluid from Human Wounds

Keyword Descriptors

IX. Project Description: Evaluation of Roentgenographic and Radioisotopic Screening Tests in the Preoperative Evaluation of the Surgical Cancer Patient

Keyword Descriptors

X. Project Description: Computer Applications to Research and Clinical Problems in the Surgery Branch

Part a. Serum Bank

Part b. Revision of Clinical Protocols

Keyword Descriptors

I. Project Description: Surgical Treatment of Malignant Melanoma

Part a. Natural History

Objectives:

To review the previous experience in this Branch with the surgical treatment of malignant melanoma in order to determine guidelines for future therapy. We have concentrated particularly on correlations between Clark's level of invasion of the primary melanoma and the incidence of lymph node metastases in palpable and nonpalpable regional lymph nodes.

Methods Employed:

Patients seen in the NCI Surgery Branch with melanoma had their slides of the primary lesion reviewed by members of the NIH Pathology Department. An attempt was made to determine the level of invasion on each primary tumor. Cases in which no primary tumor was available, or in which sections were felt to be inadequate, were eliminated. An attempt was made to consider only operable cases; therefore, cases with disseminated disease or satellitosis were excluded, and only patients undergoing prophylactic or therapeutic lymph node dissections were considered. The hospital record of all patients was reviewed to determine whether or not the patient had palpable lymph nodes prior to surgery, the type of surgery that was performed, the microscopic findings in the lymph nodes, and the ultimate outcome of the patient.

Major Findings:

We are currently analyzing 29 cases of Level II melanoma, 68 cases of Level III melanoma, 58 cases of Level IV melanoma, and 18 cases of Level V melanoma. Preliminary analysis reveals a correlation between the level of invasion and the incidence of positive lymph nodes in clinically negative regional areas. Additional correlations have yet to be made.

Significance to Cancer Research and the Program of the Institute:

We are attempting to correlate the level of invasion with the incidence of microscopic involvement in clinically negative (and positive) lymph nodes. It is hoped that this may provide additional information in deciding which patients, if any, should be subjected to prophylactic lymph node dissection.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

We plan to complete this study as outlined and use it as one of the bases for reevaluation of the Surgery Branch approach to the treatment of malignant melanoma.

Part b. Immunotherapy and Chemotherapy as Post-Surgical Adjuvants in the Treatment of Stage II Disease

Objectives:

The purpose of this study was to randomize patients with palpable positive lymph nodes for either observation, chemotherapy, or immunotherapy, following local excision and regional lymph node dissection.

Methods Employed:

Patients with palpable lymph nodes and histologic confirmation of tumor involvement were randomized to one of the three arms mentioned above. Those patients randomized for observation were followed at six week intervals in the Surgery Branch clinic. Patients randomized to the chemotherapy arm received 200 mg/m^2 of methyl-CCNU every six weeks. Patients randomized to receive immunotherapy received BCG and allogeneic tissue cultured melanoma cells. All patients were followed to the time of recurrence. Immunologic evaluation of the patients was performed serially.

Major Findings:

To date, only six to seven patients have been entered into each of the three arms, with approximately half of each group relapsing with recurrent disease. Immunologic evaluation has revealed that the initial immunizing schedule had failed to immunize patients against HL-A antigens on the injected cells, and accordingly, an increased dose and increased frequency of injections is being tested in several off-protocol patients. The data are too preliminary to draw conclusions regarding relative incidences of relapse.

Significance to Cancer Research and the Program of the Institute:

This study was designed as an initial attempt to answer the question of whether immunotherapy or chemotherapy might improve a patient's outlook following lymph node dissection for Stage II melanoma.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

This study, probably somewhat modified, will be continued in collaboration with the Immunology and Chemotherapy Branches of the NCI.

Part c. Evaluation of Staging Laparotomy

Objectives:

The objectives of this study are (1) to determine the incidence of hepatic or para-aortic melanoma involvement in patients who have disease confined to the inguinal lymph nodes, and (2) to determine the incidence of deep inguinal lymph node positivity in patients with varying degrees of positivity in the superficial inguinal lymph nodes.

Methods Employed:

Twenty-three consecutive patients with malignant melanoma of the lower extremities and documented metastases to the superficial groin, without evidence of other dissemination were studied. Each patient received multiple radioisotope and chemical studies to assess tumor dissemination. No patient had a positive liver scan. Each patient was then subjected to laparotomy. The liver was carefully palpated and/or biopsied, as were the para-aortic lymph nodes. If melanoma was found in either of these locations, the operative procedure was terminated and the patient was placed on a chemotherapy-immunotherapy protocol. If this examination of liver and para-aortic lymph nodes was negative, then the patient, during the same operation, underwent dissection of the deep inguinal lymph nodes, since each of these patients had documented metastases to the superficial inguinal lymph nodes.

Major Findings:

Three of 23 patients had positive liver or para-aortic lymph node biopsies at the time of surgery. Two of these patients had massive local disease in the superficial groin and one of the three patients had a local recurrence at the operative site at the ankle.

Twenty of the 23 patients underwent ipsilateral iliac and obturator lymph node dissection, which proved positive in 2/20 patients (10%). Patients with only microscopic superficial inguinal involvement had no hepatic or nodal spread noted at laparotomy.

Significance to Cancer Research and the Program of the Institute:

These studies indicate that patients with bulky nodal disease have, in this series, a better chance of having hepatic or para-aortic tumor dissemination compared to patients with less bulky disease. Patients with only microscopic positivity in the superficial groin had no deep iliac lymph node involvement, hepatic involvement, or para-aortic lymph node involvement.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

This information will be of value in reassessing the approach of the Surgery Branch to the surgical treatment of melanoma.

Part d. Studies on Intralesional Treatment

Objectives:

To evaluate the role of BCG, DNCB, and other agents in the intralesional treatment of malignant melanoma.

Methods Employed:

In an initial nonrandomized study on five patients, 82 lesions were injected with BCG (10^7 Tice organisms per injection), 179 lesions were injected with DNCB (50 micrograms), 35 lesions were injected with a combination of BCG and DNCB, 43 lesions were injected with acetone, the diluent for dinitrochlorobenzene, and 59 lesions were inoculated with either saline, water, or other diluents. In a second study, patients will receive only intralesional BCG or only intralesional DNCB. The first five cases have all received BCG. The next five patients will all receive DNCB, two of which have already been entered into the study.

Major Findings:

The regression rate of lesions inoculated with BCG alone was 62% (51/82). The regression rate of lesions injected with DNCB alone was 61% (110/179). The regression rate of the 35 lesions injected with BCG and DNCB, in two separate injections, was 80% (28/35). The regression rate following inoculation with acetone was 23% (10/43), and the regression rate following saline, water, etc., was 10% (6/59).

In the subsequent trial comparing BCG alone to DNCB alone, the preliminary indication is, as suggested above, that intralesional DNCB is as effective as intralesional BCG. While BCG is regularly associated with malaise, fever, and an influenza-like syndrome, these effects have not been observed following injection of DNCB.

Significance to Cancer Research and the Program of the Institute:

Throughout our studies, we have been impressed by the close correlation between local destruction by the injected agent, and the ability of that agent to destroy lesions. Superficial lesions are also more easily eradicated by any agent than deep subcutaneous lesions. For this reason, we have hypothesized that the major if not sole beneficial effect of intralesional inoculation is due to local destruction by the injected agents, without an immunological component. Also, we have been impressed with the ability of a nonreplicating agent to give equivalent regression rates and to produce the same *in vitro* change as is observed after injection of BCG, a living agent, associated with a high incidence of side effects. If DNCB is capable of producing the same *in vitro* changes as BCG, then it is also possible that the chemical agent might be substituted for BCG in studies on systemic immunotherapy as an adjuvant to treatment of solid tumors.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

We plan to complete the "alternating" series that we have started comparing intralesional BCG to intralesional DNCB. If the nonreplicating chemical agent continues to be as effective as BCG in intralesional treatment of melanoma nodules, then we feel that DNCB and not BCG should be used for this purpose, because of the dramatic difference in morbidity and complication rates following injection of DNCB. Although BCG is regularly associated with fever and malaise, we have observed no adverse effects of DNCB inoculation, except local ulceration, which occurs following either agent's intralesional use. Finally, if DNCB has the same systemic effect of enhancing lymphocyte reactivity, then it may be the agent of choice instead of replicating agents for systemic immunotherapy. These hypotheses would have to be tested in randomized trials.

Keyword Descriptors:

Melanoma, BCG, intralesional, lymph node dissection.

II. Project Description: Surgical Treatment of Sarcomas

Objectives:

To review the previous experience of the Surgery Branch with the surgical treatment of soft tissue and bony sarcomas. To develop combined modality treatments for soft tissue sarcomas and to evaluate the role of immunotherapy as a postsurgical adjuvant.

Methods Employed:

A recent total review has been performed of the Surgery Branch's experiences with soft tissue and bony sarcomas. A total of 321 sarcomas have been seen at the National Institutes of Health since 1953. The natural course of these patients is being reviewed in detail to develop information as to the natural history of this wide variety of diseases (see Table 8).

The Surgery Branch, in conjunction with the Medicine and Radiation Oncology Branches, NCI, has recently initiated a combined modality protocol for the treatment of soft tissue sarcomas of the extremities. This protocol will evaluate the need for radical surgery in achieving local control of sarcomas. Radical surgery will be compared with a combination of conservative surgery and radiotherapy for these lesions. The protocol also evaluates the usefulness of postoperative adjuvant chemotherapy and postoperative adjuvant immunotherapy.

Major Findings:

These studies are just beginning. The first patients were admitted to the new combined modality protocol in April 1975.

Significance to Cancer Research and the Program of the Institute:

The five year survival for almost all of the soft tissue and bony sarcomas is less than 40%. These results have been obtained primarily by the use of surgery without the use of other modalities. The combined modality approach to the treatment of sarcomas offers promise for improvements of the treatment of this disease. The combined modality approach to the treatment of childhood rhabdomyosarcomas has already served as the model for what can be achieved when surgery, chemotherapy, and radiotherapy are suitably combined in the treatment of sarcomas.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

The combined modality program for the treatment of sarcomas is currently in progress and will be continued.

Keyword Descriptors:

Sarcomas, adjuvant therapy, immunotherapy.

III. Project Description; Surgical Treatment of Head and Neck Cancer

Part a. Innovative Surgical Techniques for the Resection of Head and Neck Malignancies and Post-Resection Reconstruction

TABLE 8

PATIENTS SEEN AT NIH SINCE 1953

(As of January 1, 1975)

43	Fibrosarcoma
27	Liposarcoma
64	Osteogenic Sarcoma
1	Periosteal Sarcoma
45	Chondrosarcoma
1	Schwannoma
8	Leiomyosarcoma
46	Rhabdomyosarcoma
19	Synovial Cell Sarcoma
28	Undifferentiated
1	Giant Cell Sarcoma
4	Alveolar Soft Parts
14	Mesenchymoma
6	Dermatofibrosarcoma or Aggressive Fibromatosis
6	Angiosarcoma
5	Fibrous Histiocytoma
2	Myxofibrosarcoma
1	Mesothelioma
321	Total

(plus 84 Ewing's Sarcoma)

Objectives:

One of the major deterrents to the resection of advanced malignancies of the head and neck region is the resulting defect which is often so extensive that cosmetic and functional reconstruction is either impossible or is associated with numerous operative procedures with a high incidence of surgical failures. The majority of these lesions are in the mandibular and maxillary regions. We are attempting to develop techniques that facilitate operative access to these regions and improve functional and cosmetic reconstruction.

Methods Employed:

In the Surgery Branch, NCI, four such surgical techniques have been developed:

(1) Combined craniofacial resection of paranasal sinus tumors.
(2) Extended shoulder flap reconstruction of mid and upper facial defects.
(3) Extended deltopectoral flap reconstruction of composite anterior oral defects.
(4) Prosthetic reconstruction of mandible defects.

The combined cranial and facial approach to paranasal sinus cancers requires a collaboration between neurosurgeons and head and neck surgeons to approach ethmoid, sphenoid, and frontal sinus tumors by two incisions, one intracranial and the other transfacial in location. This approach allows the en bloc resection of tumors that invade the orbital walls or cribriform plate-sphenoid sinus area as opposed to the piecemeal resection commonly used after exposure of the tumor by a primarily facial incision. The extended shoulder and extended deltopectoral flaps increase the length of conventional flaps by approximately 25-35%. This extension is possible only through delays in preparation of the flap for operation. Since most patients treated for these malignancies on our service receive preoperative adjuvant chemotherapy, the time involved in the delays does not postpone resection of the tumor. Reconstruction of up to 90% of the mandible after resection of advanced tumors is facilitated by the delayed and extended deltopectoral flap which provides coverage for prostheses of this size.

Major Findings:

Thus far, the craniofacial resection has been utilized to resect tumors involving the ethmoid, sphenoid, or frontal sinuses in 54 patients. There were two postoperative deaths, both due to meningitis following resection of dura which had been invaded by tumor. A 56% five-year survival has been achieved in this group, despite the fact that 42 patients had previous surgical attempts at resection of the malignancies. These results are superior to that reported in the literature, utilizing either surgery or radiation therapy for the treatment of these tumors. The superior results obtained by this approach suggest that the achievement of an en bloc resection of the tumor is a critical factor in the high cure rates achieved in this series. Utilizing the extended shoulder flap, 14 patients have had reconstruction of frontal, maxillary, and intra-oral defects not readily reconstructed by other techniques. In all except three patients, the flap was used to reconstruct the defect primarily after tumor resection. Three to four weeks later, the flap was transected, the base returned to its bed, and the distal portion used to complete the reconstruction. The extended deltopectoral flap has been used to reconstruct 12 anterior oral defects resulting from resection of tumors involving the mandible, floor of mouth, tongue, and chin. The flaps were delayed during the interval that preoperative chemotherapy was administered, and then were rotated to reconstruct the defects at the time of tumor resection. With both flap techniques, defects beyond the reach of conventional shoulder and deltopectoral flaps were reconstructed far more quickly than would have been possible with conventional flap techniques The extended deltopectoral flap has provided intra-oral coverage for mandible implants that could not have otherwise been placed in 7 patients because of the resection of intra-oral tissues that otherwise would have been used for coverage of the implants. In all patients, the implants have provided functional and cosmetic restoration of the defects and none have required removal for any reason.

Significance to Cancer Research and the Program of the Institute:

A significant impediment to the resection of advanced tumors of the head and neck region that technically and biologically can be removed en bloc with expectation of cure rates that justify the procedure is the functional and cosmetic result after such resections. The techniques described facilitate the en bloc removal of such malignancies with adequate margins of normal tissue for avoidance of higher recurrence rates by providing techniques of wound reconstruction that lead to a relatively rapid restoration of function and appearance.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

The technique and results of the craniofacial approach to paranasal sinus cancers developed on this service have recently been described. An evaluation of the results obtained with the other techniques has recently been completed. This phase of the Surgery Branch program is currently being deemphasized.

Part b. The Use of Methotrexate as a Preoperative Adjuvant in Patients with Head and Neck Cancer

Objectives:

The objective of this study was to ascertain the influence of preoperative methotrexate on recurrence and survival in patients with operable head and neck epidermoid carcinoma.

Methods Employed:

Consecutive patients over the past 3-1/2 years with operable head and neck epidermoid carcinoma who had never been treated for their tumor prior to being

Methods Employed:

Consecutive patients over the past 3-1/2 years with operable head and neck epidermoid carcinoma who had never been treated for their tumor prior to being seen at the National Cancer Institute were included in the study. Following extensive preoperative evaluation, individuals who were found to be free of distant metastases and in whom there were no medical contraindications to the administration of systemic chemotherapy, were given 240 mg/m^2 methotrexate over 24 hours approximately 14 days prior to operative removal of their tumor. Following the initial dose of methotrexate, patients were given citrovorum-factor (leucovorin) as a rescue agent. This chemotherapy regimen was repeated 9 days prior to surgery. Patients were operated upon in the conventional manner for their tumors, with particular attention to the perioperative complications that might be attributable to methotrexate. Serial hemograms, and chemistry profiles were obtained to ascertain the effect of methotrexate on the hematologic, renal, and hepatic systems.

Thirty patients have been entered in this protocol to date and analysis of the survival and recurrence data of this group was compared to a group of 30 retrospective historical controls treated at the National Cancer Institute. Controls were matched for tumor extent and histology. Each control has been followed for at least 3 years or until death.

Major Findings:

Methotrexate in high doses described by this protocol has been found safe and to not complicate the operative procedure or the postoperative management of these patients. Methotrexate prolonged the disease-free interval significantly; however, it did not change the ultimate number of patients who develop recurrent tumor. When the survival data was analyzed in this group of patients, a similar trend was seen. The methotrexate-treated patients ultimately died, on the average, later than those in the control group; however, further follow-up is necessary for adequate statistical analysis.

Significance to Cancer Research and the Program of the Institute:

This work confirms that a chemotherapeutic agent given as a preoperative adjuvant can improve the natural course of disease in patients operated on for squamous cell carcinoma of the head and neck by delaying recurrence. While no significant alteration in the ultimate survival or recurrence data has been achieved with this regimen, it is possible that greater benefit could be obtained with higher doses, combinations of chemotherapeutic agents, or different single agents alone.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

The patients entered in this study will continue to be followed until complete follow-up is obtained. In the interim, selected patients will be added to expand the study group. The possibility of adding other agents to the preoperative regimen or increasing the dosage of methotrexate is being considered.

Keyword Descriptors:

Head and neck cancer, methotrexate, reconstructive surgery.

IV. Project Description: Surgical Treatment of Carcinoma of the Cervix

Objectives:

To evaluate extended surgical procedures for treatment of patients with recurrent cervical cancer and for selected patients with primary carcinoma of the uterine cervix.

Methods Employed:

Approximately 2100 patients with cancer of the uterine cervix have been referred to the Surgery Branch for evaluation in the past 20 years. From this large group, 550 were admitted for inpatient evaluation, 406 of whom underwent abdominal exploration with plans for definitive surgical therapy.

Of the patients explored, 163 were not candidates for exenterative surgery because of the presence of extrapelvic lymph node metastasis, intraperitoneal spread, or disease fixed to the bony pelvis. Eighty-two patients had relatively early cancers and could be effectively managed with a radical hysterectomy and pelvic lymphadenectomy. Thirteen of these patients had small radiorecurrent lesions, the remainder had primary cervical cancer.

One hundred sixty-one patients required either partial or total pelvic exenteration for control of their disease.

Major Findings:

The major points of interest are summarized in Tables 9, 10, and 11. The 5-year actuarial survival of those treated with radical hysterectomy and pelvic lymphadenectomy was 83.5%. Those patients with radiorecurrent or persistent disease survived at a rate similar to those with primary carcinoma. The presence of metastatic carcinoma in the pelvic lymph nodes adversely affected prognosis. The 5-year actuarial survival for those treated with exenteration of the pelvic viscera was 38%.

Significance to Cancer Research and the Program of the Institute:

The results obtained with extended surgical procedures are highly favorable and surpass those reported from other institutions (Annual Report on the Results of Treatment of Carcinoma of the Uterus and Vagina, 14th Volume). Extended surgical procedures are demonstrated to be effective in controlling advanced cervical cancer and are the only effective treatment modalities for

TABLE 9

CARCINOMA OF THE CERVIX: ACTUARIAL 5-YEAR SURVIVAL FOLLOWING TREATMENT WITH EXTENDED SURGICAL PROCEDURES

OPERATION PERFORMED	PRIMARY CARCINOMA PELVIC LYMPH NODES			RECURRENT CARCINOMA PELVIC LYMPH NODES		
	Positive	Negative	Total	Positive	Negative	Total
Radical Hysterectomy and Pelvic Lymphadenectomy	60.0%	91.3%	82.8% (69)*	85.7%	83.3%	84.6% (13)
Pelvic Exenterations (Partial and Total)	54.2%	52.2%	52.6% (66)	18.4%	35.6%	31.8% (95)
TOTAL (All extended surgical procedures without resection of bony pelvis)	56.4%	74.4%	67.6% (135)	30.5%	39.9%	39.1% (109)

*Numbers in parentheses represent the number of patients studied.

TABLE 10

CORRELATION OF THE MEIGS' SURGICAL PATHOLOGIC STAGING WITH SURVIVAL OF 162 PATIENTS WITH CARCINOMA OF THE CERVIX WHO UNDERWENT PELVIC EXENTERATION

	5-YEAR CUMULATIVE PERCENT SURVIVAL		
PATHOLOGIC STAGING	PRIMARY CASES	RECURRENT CASES	ALL CASES
$A + A_o$	75% (5)*	43% (7)	60% (16)
B	56% (10)	0% (14)	33% (24)
C	31% (11)	49% (25)	44% (36)
D	60% (10)	17% (6)	44% (16)
E	43% (32)	18% (38)	30% (70)

*Numbers in parentheses represent total cases in each group.

TABLE 11

PRIMARY CARCINOMA OF THE CERVIX: ACTUARIAL SURVIVAL (BY STAGE) FOLLOWING EXTENDED SURGICAL PROCEDURES

	PELVIC LYMPH NODES		
	Positive	Negative	Total
Stage I	75%	87%	84.3% (37)*
Stage II	60%	86%	80.0% (42)
Stage III	50%	75%	60.0% (10)
Stage IV	50%	32%	39.5% (37)

*Numbers in parentheses represent the number of patients studied.

patients with radiorecurrent cervical cancer. Selected individuals are offered vaginal reconstruction in an attempt to contribute to complete rehabilitation.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

The limits and the values of extended surgical procedures for advanced carcinoma of the cervix have been defined. This phase of the Surgery Branch program is currently being deemphasized.

Keyword Descriptors:

Cancer of cervix, exenteration.

V. Project Description: Surgical Studies in Breast Cancer

Part a. Detection of Early Breast Cancer

Objectives:

One hope for improvement in the treatment of breast cancer lies in diagnosing and treating the disease at a time when deep infiltration and widespread dissemination are least likely to be present. To this end a variety of diagnostic procedures are being evaluated in the Breast Problem Clinic of the Surgery Branch. Patients with breast masses, nipple discharge or abnormal breast x-rays are accepted for evaluation.

Methods Employed:

In addition to a detailed history and thorough physical examination, particular emphasis is put on detection of breast disease by use of conventional mammograms and breast xerograms, isotope scanning of the breasts with Technetium Pertechnetate, and serum and urine chemical markers.

In the past 12 months the Surgery Branch performed 51 breast biopsies for suspicious findings on one of the above studies.

Major Findings:

In a study of isotope scanning in cooperation with the Nuclear Medicine Department the scan was interpreted as abnormal in 14 out of 16 patients with breast masses that were later diagnosed as cancer. In contrast to the 88% true positive result, however, 29% of proven benign breast disease also had abnormal scans. Although this would limit the usefulness of such scanning for detection of breast cancer, modification of technique may prove helpful in the future.

Blood and urinary markers were done routinely on patients undergoing work-up for possible breast cancer. These studies showed that one or more of the three tests, namely, CEA, HCG, and N^2, N^2-dimethyl-guanosine were abnormal in 97% of the patients with metastatic disease and in 67% of the patients with positive lymph node involvement. While these tests are nonspecific for breast cancer, continued study of other markers may be more rewarding.

Specific protocols being pursued to answer some of the questions concerning the patient with early breast carcinoma are (1) Protocol B-13A, The Evaluation of Tumor Markers in Patients with Breast Cancer, (2) Protocol B-13B, Evaluation of Hormone Levels in Patients with Breast Cancer, (3) Protocol B-13C, Evaluation and Prognosis Usefulness of Barr Body Evaluation in Breast Cancer, and (4) Protocol B-13D, Evaluation of Chromosomes in the Patients with Breast Cancer.

Significance to Cancer Research and the Program of the Institute:

The aim of the multidisciplinary approach to the early diagnosis of breast cancer through screening and identification of a population at risk should improve the survival of patients who may still be curable from this disease and will lead to a better understanding of the natural biology of breast cancer.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

Expansion and further development of currently instituted protocols and the development of new protocols aimed at improving the early detection of breast cancer.

Part b. Treatment of Primary Breast Cancer

Objectives:

Evaluation of postoperative chemotherapy and immunotherapy in the treatment of patients with cancer of the breast.

Methods Employed:

Through the combined efforts of the Medical Breast Cancer Section of the Medicine Branch and the Breast Oncology Section of the Surgery Branch, patients who are found to have suspicious lesions undergo biopsy and, if positive, modified radical mastectomy. Sixteen modified radical mastectomies have been performed in the last year, and depending upon the patient's nodal status and stage of disease, have been entered into other protocol studies evaluating adjuvant therapy. Twenty-eight patients who have had their surgery here and elsewhere have been entered into a combined modality approach protocol evaluating the use of adjuvant chemotherapy and/or chemotherapy and immunotherapy. The patients with negative axillary nodes are being followed during the disease-free interval and are evaluated periodically for evidence of metastatic disease as well as the possibility of new primary cancers in the opposite breast. Serum markers and radioisotope scans are included in these studies. Biopsy material from the nonmalignant breast tissue and tumor are assayed for hormonal receptor, estrogen, androgen, progesterone, and cortisone.

The Surgery and Medicine Branches are collaborating in Protocol BTS-135, Evaluation of a Combined Modality Approach to the Therapy of Mammary Carcinoma in Patients with T1, 2, 3A, and 4B with N(+) Lesions. This is the basic protocol for adjuvant chemotherapy of surgically treated patients who have positive axillary nodes. Such patients are currently being randomized between chemotherapy and chemotherapy in combination with immunotherapy.

Major Findings:

Twenty-six patients have thus far been entered on these studies with randomization of 15 to receive L-PAM, 10 to the CMF arm, and 3 in the immunotherapy plus CMF arm.

Significance to Cancer Research and the Program of the Institute:

The aim of the multidisciplinary approach to the treatment of breast cancer is the appraisal of current treatment modalities and their improvement by the use of adjuvant techniques. Also the procurement of material for biologic assay will lead to a better understanding of the nature of breast cancer.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

It is hoped to further expand and develop currently instituted protocols for the treatment of primary breast cancer.

Part c. Treatment of Metastatic Breast Cancer

Objectives:

To improve the treatment of patients with metastatic breast cancer and to develop more reliable methods of identifying patients who may be helped by endocrine ablation.

Methods Employed:

Through the combined efforts of the Medicine Branch and the Surgery Branch, patients with metastatic breast cancer are processed through a series of interlocking treatment protocols that include palliative resections, endocrine ablation, and chemotherapy. These are illustrated by (1) BTS-122, A Study to Evaluate the Effect of Cyclophosphamide, Methotrexate, and 5-Fluorouracil versus Cyclophosphamide, Adriamycin, and 5-Fluorouracil. This study currently is evaluating eligible patients who have not received previous chemotherapy but who present with, or are referred with, metastatic breast cancer. (2) BTS-123, Study to Evaluate the Effect of Oophorectomy with and without Adjuvant

Chemotherapy in the Management of Recurrent Mammary Carcinoma in Premenopausal Women. In the past year three patients have been entered into this study, having had their oophorectomy performed by the Surgery Branch. (3) Protocol BTS-124, Study to Evaluate the Effect of Estrogen Therapy with and without Additive Chemotherapy in the Management of Recurrent Mammary Cancer in Postmenopausal Women. (4) Protocol BTS-143, designed to study the effect of multiple agent chemotherapy as compared to multiple agent chemotherapy given in a sequentially altering fashion. (5) Protocol B-151, The Prospective Use of Steroid Hormone Receptor in Predicting the Response to Endocrine Manipulation in Metastatic Breast Cancer.

Major Findings:

These studies have just begun and data is being accumulated.

Significance to Cancer Research and the Program of the Institute:

Information being accumulated will allow the application of the most appropriate therapy to the individual patient with metastatic breast cancer based on a better understanding of the individual biologic characteristics of the tumor. New treatment modalities are being evaluated in an attempt to prolong survival of patients with metastatic breast cancer.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

It is planned to expand and further develop currently instituted protocols and studies with particular emphasis on the development of new protocols within the Surgery Branch.

Part d. Treatment of Male Breast Cancer

Objectives:

The purpose of these studies is to evaluate the hormone receptor status and serum and urine hormone alterations prior to and subsequent to endocrine ablations in male patients with breast cancer.

Methods Employed:

Males with breast cancer have their tumor assayed for hormone receptors; those with metastatic or recurrent tumor undergo orchiectomy and, upon failure, subsequent adrenalectomy. Urine and serum hormonal alterations are then correlated with changes in the amount of receptor activity in the tumor and with response to endocrine ablative therapy.

Major Findings:

Preliminary investigations have demonstrated a very high proportion of male breast cancers that contain hormonal receptors. Androgen, estrogen, or progesterone receptors may be present; some tumors have all three. Retrospective analysis of males treated at the NIH demonstrates a remission rate of over 60% to orchiectomy alone (Table 12). This correlates well with other reported series and is considerably greater than the response rate of females with breast cancer to endocrine ablations.

Significance to Cancer Research and the Program of the Institute:

This study will further evaluate the usefulness of hormone receptors in hormonally dependent tumors; the possibility of selecting those patients who will respond to endocrine manipulations as based on the hormonal receptors in their tumor will be further investigated. Evaluating the serum and urine hormone alterations following endocrine manipulations may allow early identification of the recurrence of a tumor. Male breast cancer is of particular interest because its remission rate to endocrine manipulations is higher than that for any other hormonally dependent tumor.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

This study is in its early stages. Additional patients must have their tumors studied for hormonal receptors and then have those values correlated with response to therapy; additional patients must have serum and urine hormonal alterations analyzed to determine their possible usefulness as markers.

Keyword Descriptors:

Breast cancer, hormone receptors, adjuvant therapy.

VI. Project Description: Genitourinary Aspects of Cancer Management

Part a. Study of Urinary Diversion in Pelvic Malignancy

Objectives:

Although the ileal conduit is widely used as a method of supravesical urinary diversion, there are significant short-term and long-term complications. These complications have included stomal stenosis, especially in the patients who have received previous irradiation, and renal failure secondary to infection due to free ureteral reflux. In order to avoid stomal stricture, urinary tract infection and renal failure, a technique of urinary diversion utilizing a loop of sigmoid colon to construct an anti-reflux ureteral sigmoidoscopy is being evaluated in the Surgery Branch.

Methods Employed:

After appropriate preoperative investigation of the sigmoid colon, a 10 cm sigmoid colon is isolated. The proximal portion of the sigmoid is closed and

TABLE 12. PATIENTS UNDERGOING ORCHIECTOMY FOR ADVANCED BREAST CARCINOMA

Pt.	Age at Dx.	Primary Rx.*	Date Primary Rx.	Date Orch.	Resp. to Orch.	Recurr.	Dis. Free Interval (months)	Follow-up
C.G.	52	S.M.	1945	1/53	None		0	d. 8/54
R.B.	61	S.M.	1951	11/55	None		0	d. 11/57
G.S.	66	S.M.	1959	9/59	Healing soft tissue	9/61	24	d. 9/63
A.N.	37	R.M.	1956	12/58	Healing bone	11/60	23	d. 9/65
W.B.	68	S.M.	4/53	10/59	Healing of pulm., soft tissue progression	10/60 - pulm. recurr.	0	d. 11/62
J.W.	43	R.M.	7/51	12/59	Healing of pulm. mets.	10/61	22	d. 4/66
D.E.	65	R.M.	6/61	12/62	Healing of bone and soft tissue	6/64	18	d. 9/65
C.K.	26	B.R.M.	5/66	8/69	Healing of bone	9/70	13	d. 1/73
M.S.	64	M.R.M.	6/74	8/74	Too early to evaluate			

*S.M. - Simple mastectomy
R.M. - Radical mastectomy
B.R.M. - Bilateral radical mastectomy
M.R.M. - Modified radical mastectomy

the distal portion is brought out as the stoma. A ureterosigmoidostomy is performed by a submucosal tunnel technique in order to prevent reflux of the contents of the sigmoid conduit.

Major Findings:

The use of sigmoid conduits seems to be a safe alternative to the ileal conduit for urinary diversion in patients with pelvic malignancies requiring permanent urinary diversion. The course of 11 patients with sigmoid urinary diversion following total pelvic exenteration or anterior pelvic exenteration have supported these findings (see Tables 13 and 14). A cine-radiologic study of the sigmoid conduit has revealed proper emptying of the conduit and the absence of reflux into the ureter. Preliminary data reveals a low frequency of infection in these patients. The frequency of infection in 250 ileal conduits which have been performed in the Surgery Branch in the past has been over 40% in spite of active or suppressive antibacterial therapy.

Significance to Cancer Research and the Program of the Institute:

The use of the anti-reflux sigmoid conduit appears to offer many advantages compared to the ileal conduit. This procedure may reduce the morbidity and mortality of the patient with pelvic malignancy requiring urinary diversion.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

This project will continue to evaluate the long-term advantages and possibly disadvantages of sigmoid conduit urinary diversion.

Part b. Management of Massive Bladder Hemorrhage Secondary to Cyclophosphamide or Radiation Therapy in Cancer Patients

Objectives:

Acute hemorrhagic cystitis following radiation therapy or treatment with cyclophosphamide for various lymphoproliferative malignancies and selected solid tumors has been relatively common in the National Cancer Institute. Bleeding is occasionally massive and life-threatening, and despite the frequent use of multiple transfusions and other conventional modalities of treatment, the morbidity and mortality have been significant. The use of formaldehyde for local instillation in the bladder to treat massive bladder hemorrhage is being evaluated.

Methods Employed:

The safety and adequacy of formaldehyde instillation into the bladder was first evaluated in animals. It was then applied to 6 patients with life-threatening bladder hemorrhage refractory to the conventional treatment techniques.

TABLE 13

COLONIC CONDUITS

PATIENT		AGE (YEARS)	TUMOR	FOLLOW-UP (MONTHS)
1.	GM	72	Neurogenic bladder	4
2.	FM	60	Sq. Cell Ca Cx[+]	5
3.	MC	29	Sq. Cell Ca Cx	6
4.	BM	53	Tr. Cell Ca Bl[*]	7
5.	RH	40	Sq. Cell Ca Cx	16
6.	AW	37	Sq. Cell Ca Cx	19
7.	GB	57	Sq. Cell Ca Cx	21
8.	BB	33	Sq. Cell Ca Cx	22
9.	NR	60	Sq. Cell Ca Cx	32
10.	EJ	44	Sq. Cell Ca Cx	38
11.	MR	52	Sq. Cell Ca Cx	225

[+] Squamous cell carcinoma, uterine cervix.

[*] Transitional cell carcinoma, bladder.

TABLE 14

COMPLICATIONS FOLLOWING SIGMOID CONDUIT URINARY DIVERSION IN ELEVEN PATIENTS*

		COLONIC
A. Urinary Complications		
Leakage	- anastomosis	0[+]
	- conduit	0
Obstruction	- anastomosis	0
Conduit	- necrosis	0
	- redundancy	1
Stoma	- stenosis	1
	- hemorrhage	0
	- retraction	0
	- hernia	1
	- skin disorder	1
B. Gastrointestinal Complications		
Small bowel obstruction		3
Fistula	- enteric	1
	- fecal	0
	- enteroconduit	0
C. Renal Complications		
Azotemia		0
Progressive renal failure		0
Chronic pyelonephritis		1
D. Metabolic Abnormalities		
Hyperchloremia		1
Chemical acidosis		2
Hypokalemia		2
Alkaline urine		2
E. Infectious Complications		
Stomal abscess		0
Acute pyelonephritis		2
Treated urinary infections		5
Urine culture $>10^5$ org/ml		8

*Total number of patients.

[+] Number of patients with each complication.

Major Findings:

Cyclophosphamide-induced changes in the bladder are (a) atypia of transitional cells with ulceration of the mucosa, (b) massive submucosal talengectasia, (c) fibrosis of the detrusor muscle leading to compromised function of the bladder. These changes have been observed in patients suffering from cyclophosphamide bladder toxicity as well as radiation toxicity and have been confirmed in mice and dogs by experimental induction. The pre- and post-formaldehyde instillation biopsies have revealed no significant changes after formaldehyde therapy. We have successfully utilized this type of treatment in six patients with recurrent intractable gross hematuria refractory to conventional therapy. Six to 12 month follow-up of these six patients has revealed no bad side effects. One patient required a second instillation of refined formaldehyde.

Significance to Cancer Research and the Program of the Institute:

These studies have demonstrated an alternative therapy for treating intractable cyclophosphamide cystitis with massive bleeding. The lack of complications and side effects of refined formaldehyde in the bladder and the effectiveness of hemostasis has encouraged us to continue this program.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

We plan to continue this study and follow the patients who have received this treatment with bladder biopsy, cystometrogram, and other functional studies in order to evaluate the long-term safety and effectiveness of this modality of treatment.

Part c. Tumor Markers in Patients with Testicular Germ Cell Tumors

Objectives:

Since the early detection of recurrence of testicular tumors after therapy is difficult with conventional procedures, we have undertaken a study of the value of tumor markers such as human chorionic gonadotropin, alpha-fetoprotein, and carcinoembryonic antigens in monitoring the course of patients with germinal cell testicular tumors and predicting the response to therapy.

Methods Employed:

Tumor markers including human chorionic gonadotropin, alpha-fetoprotein, and carcinoembryonic antigens have been measured by radioimmunoassay. These tumor markers have been correlated with clinical response to treatment and the presence or absence of tumor extension or metastases.

Major Findings:

The use of a radioimmunoassay has revealed the presence of chorionic gonadotropin in over 50% of patients with germ cell testicular tumors. We have found that 80% of the patients with teratocarcinoma and embryonal carcinoma have elevated alpha-fetoprotein. The results of radioimmunoassays of these markers in 81 patients with different types of testicular tumors are tabulated in Tables 15 and 16.

Significance to Cancer Research and the Program of the Institute:

Using the radioimmunoassay technique to measure chorionic gonadotropin, over 50% of the patients with testicular tumors have a significant level of chorionic gonadotropin. With conventional techniques, only 10% of the patients exhibited the presence of chorionic gonadotropin. Since alpha-fetoprotein is elevated in patients with hepatoma and germ cell tumors, these markers may be of value in patient monitoring and predicting the response to therapy in patients with testicular tumors.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

These assays will be correlated with the course of the patient and the recurrence of the tumor. Data accrual will continue.

Keyword Descriptors:

Sigmoid conduit, cyclophosphamide, tumor markers, testicular tumors.

VII. Project Description: Endoscopic Studies

Part a. Laparoscopy as a Staging Modality for Patients with Malignant Disease

Objectives:

Appropriate initial therapy for patients with malignant disease is predicated on the ability to accurately assess the extent of tumor dissemination at the time of clinical presentation. The experience with, and success in, the treatment of Hodgkin's disease emphasizes this fact. The objective of this study is to explore the efficacy and estimate the accuracy of the laparoscope in determining the presence or absence of hepatic metastases and/or peritoneal involvement in several different malignancies.

Methods Employed:

Diagnostic laparoscopy has been used in the Surgery Branch for the first time this year. Suitable patients undergo laparoscopy as a part of their total clinical evaluation. The examination is performed in the operating room, under

TABLE 15

TUMOR MARKERS IN TESTICULAR TUMORS

Total Number of Patients	81
Seminoma	17
Teratocarcinoma	12
Embryonal Cell Carcinoma	45
Choriocarcinoma	1
Other	6

TABLE 16

RESULTS OF RADIOIMMUNOASSAYS

Cell Type	HCG		AFP		CEA	
	+	-	+	-	+	-
Seminoma	0/4	4/4	0/9	9/9	4/10	6/10
Teratocarcinoma	1/8	7/8	2/8	6/8	2/4	2/4
Embryonal Cell Ca	8/19	11/19	14/28	14/28	2/15	13/15
Choriocarcinoma	---	---	---	---	---	---
Other	0/3	3/3	0/3	3/3	1/1	0/1
TOTAL	9/34 (26%)		16/48 (33%)		9/30 (30%)	

general anesthesia, with a Storz laparoscope. Careful inspection of the peritoneal cavity and, where indicated, forceps or needle biopsy of suspicious tissue is performed. Visual and histologic findings are then used as a guide to definitive therapy.

Major Findings:

Six patients have undergone laparoscopic evaluation to date. This group includes patients with melanoma, soft tissue sarcoma, and pancreatic cancer. Two patients were found on laparoscopy to have intraperitoneal dissemination or liver metastases. The procedure was well tolerated by the patients. No morbidity or mortality was incurred.

Significance to Cancer Research and the Program of the Institute:

By permitting the accurate assessment of tissues only poorly evaluable by present laboratory, roentgenographic, and radioisotopic scanning techniques, this minimally invasive endoscopic procedure promises to spare patients a major diagnostic abdominal exploration. The staging information so gained will insure appropriate tailoring of therapy to meet clinical patient needs and at the same time provide important prognostic information.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

A rectal cancer protocol, designed to compare abdominoperineal resection with electrofulguration, is currently being developed. This protocol will incorporate laparoscopy in an effort to delineate the group of patients presenting with Stage D disease (hepatic metastases). Because they are surgically incurable, these patients (probably 30% of the presenting group) will be treated by electrofulguration of their rectal tumors rather than abdominoperineal resection and colostomy. A critical effort will be made to determine the proportion of false negative laparoscopic examinations. Inspection and palpation of the liver at laparoscopy (in Stage A, B, and C patients randomized to abdominoperineal resection) will be used to confirm or refute the laparoscopic findings.

When appropriate, laparoscopy will remain an adjunctive part of the diagnostic evaluation of patients with a variety of other tumors.

Part b. Colonoscopy

Objectives:

To evaluate the role of colonoscopy in the diagnosis and management of patients with suspected colon malignancy. To test in a prospective, controlled, clinical manner, the hypothesis that saline lavage of the GI tract will offer significant advantages over current preoperative procedures for patients undergoing sigmoidoscopy and colonoscopy.

Methods Employed:

In the past 12 months 28 patients underwent diagnostic colonoscopy. Patients between the ages of 18 and 70 without cardiovascular disease, without difficulty swallowing, and free of mental impairment or disease of the GI tract that contraindicate traditional cancer evaluation, were subjected in a double-blind fashion to either the conventional preparation or saline lavage. Patients received between 2-5 liters of isotonic salt solution with potassium and bicarbonite added, administered over a 1 - 1-1/2 hour period.

Major Findings:

The endoscopic surgeon was unaware of the patient's preparation and graded the preparation as to the amount of fluid in the colon, amount of mucosal irritation and the amount of spasm encountered. To date, approximately 14 patients have been randomized to one group or the other and the code has not been broken.

Significance to Cancer Research and the Program of the Institute:

Hopefully this study will delineate a technique of preparing patients which will be less time consuming and more agreeable to the patient. This may allow endoscopic studies for the detection of cancer to be performed in greater facility and in a larger number of patients. In so doing, perhaps cancer may be detected in an earlier stage or treated by endoscopic techniques rather than by conventional surgical techniques.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

To randomize at least 50 patients in each group before breaking the code, following which the results will be evaluated.

Part d. Mediastinoscopy in Patients with Carcinoma of the Breast

Objectives:

Recent investigations have demonstrated that the response of a patient with disseminated breast cancer to hormonal manipulations is dependent on the estrogen receptor status of the tumor. Many patients who present with metastatic carcinoma of the breast do not have easily accessible tumor for biopsy; accordingly, methods for discovering sites of metastases where sufficient tumor is present for estrogen receptor analysis are needed.

Methods Employed:

Total body gallium - 67 scintiscans were performed on all patients admitted to the Medical Breast Cancer Service; these were correlated with chest x-rays and mediastinal tomograms. Patients with mediastinal abnormalities then underwent mediastinoscopy if there was no evidence of other more easily accessible tumor.

Major Findings:

Six patients have undergone mediastinoscopy to obtain tissue for estrogen receptor analysis: Five had abnormal gallium scintiscans and three had hilar adenopathy on x-ray. All patients had tumor documented by histologic and/or estrogen receptor analysis. Four other patients had abnormal gallium scintiscans and two other patients had hilar adenopathy but did not undergo mediastinoscopy; their clinical course was consistent with the presence of mediastinal metastases. Follow-up gallium scintiscans were useful as an aid in the evaluation of a response to therapy. Five of six patients undergoing mediastinoscopy had estrogen receptor activity present. There were no false positive gallium scans, but the absence of an abnormality by scintigraphy did not eliminate the possibility of mediastinal metastases as demonstrated by roentgenography and subsequent mediastinoscopy.

Significance to Cancer Research and the Program of the Institute:

This study demonstrates that gallium scintigraphy and mediastinal tomography can be employed to discover mediastinal metastases of breast cancer. These metastases are accessible for biopsy via mediastinoscopy; sufficient tissue may be procured to evaluate the tumor for estrogen receptor analysis and select out those patients who will not respond to endocrine manipulations.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

Gallium scintigraphy is to be used in those patients being evaluated for mediastinal metastases. It is not a useful screening test for other sites of metastases. Mediastinoscopy will continue to be utilized only in those patients with mediastinal abnormalities by scintiscan or tomography who do not have other areas of more easily accessible tumor.

Keyword Descriptors:

Laparoscopy, colonoscopy, mediastinoscopy.

VIII. Project Description: Microbiologic Studies in Cancer Patients

Part a. Changes in Bacterial Colonization in the Surgical Cancer Patient

Objectives:

There has been an apparent increase in serious gram negative infections in hospitalized and particularly in cancer patients in the past several years. The local factors potentially contributing to this increase have not been clarified and are the subject of these studies. Although antibiotics have been shown to be of value in preventing postoperative infections, their contribution to the selection of persistent resistant organisms is unknown and is being evaluated.

Methods Employed:

All patients entering the Surgery Branch, NCI, for definitive surgical treatment of a malignancy were eligible for the study. Qualitative bacterial cultures were obtained from eight sites: anterior nares, oropharynx, standard skin site, incision, rectum, vagina, urine, and tumor when available. These were obtained at each of six intervals: admission, one day preoperatively, and 2, 7, 14, and 21 days postoperatively. Patients were randomized into two groups. The first received cephalothin pre-, intra-, and for one day postoperatively. The second group received the antibiotic pre-, intra-, and for one week postoperatively. The dosage was 1 gram intravenously every six hours.

Bacterial changes, sensitivities to the antibiotic, and infections were followed.

Major Findings:

This study has been partially completed with initial culture of 89 patients of whom 53 were suitable for inclusion in the study and subsequent culture. The data are presently being analyzed.

A previously completed preliminary study which included six patients given the one week antibiotic regimen demonstrated an increase in resistant gram negative organisms culturable from the patients. There was an increase, particularly in the Enterobacter species. With such small numbers, the results were not statistically significant. It is unknown how these observations will be reflected in the larger study.

Significance to Cancer Research and the Program of the Institute:

This study may help to define the bacterial ecological pressure exerted by antibiotic administration. It will help to clarify the optimal period for postoperative administration of antibiotics prophylactically.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

Comparisons should be made to bacterial changes occurring in surgical patients not treated with antibiotics. This phase of the Surgery Branch program is being deemphasized.

Part b. Antibiotics in Tissue Fluid from Human Wounds

Objectives:

To be effective, an antibiotic must reach the site of the infective process. In the preventive use of antibiotics in surgical patients, the antibiotic must therefore reach the surgical wound. The dosage and administration schedule for optimal wound levels of many common antibiotics remains unknown. This study was designed to determine the concentration of antibiotic in fluid draining from human surgical wounds.

Methods Employed:

All surgical patients treated at the NCI who underwent regional lymph node dissection for malignant disease were eligible for this study. Routine treatment in these cases includes a closed suction drainage system with placement of catheters into the wound at operation. Antibiotics were given intravenously every six hours. The dosage of cephalothin was 1 gram; cefazolin 500 mg; cephapirin 1 gram; and clindamycin 300 mg. Wound catheters were maintained on constant suction with intermittent manual aspiration. During the first postoperative day, samples of the aspirate from the catheters were collected at 1.5, 3, and 5 hours after antibiotic administration. Blood samples were collected at 1.5, and 3 hours. Tissue fluid samples were frozen in liquid nitrogen for later bioassay of antibiotic concentration by three participating drug companies on a blind basis.

Major Findings:

Forty patients have completed the study. Of these, 16 received cephalothin, 9 cefazolin, 8 cephapirin, and 7 received clindamycin. The results show the patterns of appearance of antibiotics in the tissue fluid and the levels achieved. The blood level of cephalothin dropped to less than 10 mcg/ml by 1.5 hours with none detectable after 5 hours. The concentration in the tissue fluid was higher at all times (12 mcg/ml at 1.5 hours, and 1 mcg/ml at 5 hours). The blood level of cefazolin was 22 mcg/ml at 1.5 hours and 10 mcg/ml at 5 hours. The concentration of antibiotic in the initial samples of tissue fluid was lower than the serum. It surpassed the serum levels after 2-1/2 hours, reaching a peak of 15 mcg/ml at 3 hours, followed by a slow decline to 10 mcg/ml at 5 hours. (It should be noted that a level of 10 mcg/ml is sufficient to inhibit approximately 90% of gram negative organisms susceptible to the cephalosporins.) Samples from patients on cephapirin and clindamycin are presently being assayed.

The results indicate that therapeutic levels of antibiotics can be achieved in human wound fluid after the dosages employed. Tissue fluid concentration may be higher than serum concentration.

Significance to Cancer Research and the Program of the Institute:

These studies demonstrate that these antibiotics, when used on a preventive basis in surgical cancer patients, can achieve therapeutic levels within the surgical wound. They permit design of dosage schedules to maximize benefit with minimal dosage and exposure of the patient to antibiotics.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

Additional drugs may be studied. Comparative dosage schedules may be evaluated to define those providing maximal tissue level.

Keyword Descriptors:

Microbiology, antibiotics, surgical infections.

IX. Project Description: Evaluation of Roentgenographic and Radioisotopic Screening Tests in the Preoperative Evaluation of the Surgical Cancer Patient

Objectives:

Accurate staging is essential in the preoperative evaluation of cancer patients in order to define the extent of disease and to plan appropriate surgical or medical therapy. A variety of radiologic examinations and radioisotopic scans have been used for such staging evaluations of all types of cancer patients. However, the values of these examinations vary with regard to the accuracy in defining both the size and nature of primary tumors and the existence of secondary metastatic tumors. The overall sensitivity of the various screening tests are unknown, and the relative values of the tests in detecting disseminated malignant disease have not been determined. The purpose of this project is to review the five-year Surgery Branch experience with various screening tests in order to define their accuracy and to delineate those most appropriate for each of the various tumors studied.

Methods Employed:

In the past, cancer patients admitted to the NCI Surgery Branch have undergone a preoperative core evaluation. Screening tests have included: chest x-ray with full lung tomograms; excretory urography; upper and lower gastrointestinal series; metastatic bone survey; radioisotopic scans of brain, bone, liver, and total-body gallium; and blood chemistry panel including hepatic enzymes. Scalene node biopsies were performed in selected patients.

Review was performed of the records of 566 patients admitted to the NCI Surgery Branch during the five year period 1969 to 1974. Notation was made of the results on admission of various screening tests. For all patients the disease stage and subsequent clinical course were recorded. Findings of the various screening examinations were correlated to actual pathologic findings and to clinical course. Specifically, attention was directed to the relative efficacy of the various tests in predicting extent of disease and in detecting the presence of occult metastases. Similar criteria for the evaluation of extent of malignant disease were utilized for the assessments of all the screening tests. Metastatic disease detected by a positive screening test was considered present only if confirmed by biopsy, autopsy, or by subsequent clinical progression. Similarly, a suspicious lesion on any screening test was considered to be a false positive result if the lesion was subsequently proven benign on biopsy or by lack of clinical progression. False negative screening tests were detected by the subsequent clinical appearance of metastatic disease within three months of a normal screening test. Comprehensive follow-up permitted accurate evaluation of almost all patients.

Major Findings:

Screening by chest x-ray and full lung tomography yielded the following results: On screening for occult pulmonary metastases by standard chest roentgenography, 37 patients were detected as having metastatic malignant disease to the lungs. This total represented 63% of the total patients (59) having pulmonary metastases confirmed on lung biopsy, autopsy, or clinical follow-up. Five patients (8.5% of those with confirmed metastases) had x-rays read as suspicious but equivocal for pulmonary lesions. Fifteen patients with confirmed metastases had negative chest x-rays, giving a nondetection rate of 25% by chest roentgenography alone. Screening the same patients by full lung tomography detected metastases in 56 patients (95% of those with confirmed metastases). In 14 of these individuals, the routine chest x-rays were normal. It was concluded that chest tomography is a significantly more sensitive screening measure than routine chest roentgenograms for the detection of pulmonary metastases.

Review of the results of patient screening by radioisotopic scans revealed the following information: There were 326 patients who had liver scans which could be evaluated by appropriate follow-up. When reviewing these 326 scans it was found that 92% were negative, 4% were falsely positive for metastatic disease to the liver, 2% were truly positive for hepatic metastases and 2% falsely negative. The liver scan was, therefore, felt to be an accurate test but in general having a low yield, except in selected diseases and circumstances. In patients with Stage II and Stage III melanoma, the yield was higher and it is felt that the liver scan would be of value. In pelvic neoplasms there were positive scans and patients with metastatic disease to the liver, but the exact value of these scans in these patients is still to be interpreted. Among patients with head and neck cancer and in sarcoma patients there were no hepatic metastases. Therefore, the liver scan was only confusing by virtue of several false positive scans. Bone scans and metastatic series were also reviewed as well as brain and gallium scans. The brain scan was found to be of little use as a screening test for the cancer surgery patients. One hundred seventy brain scans could be evaluated in surgically operable patients. There was only one positive brain scan with confirmed intracranial pathology. There were four false positive brain scans. It is felt the brain scan should be reserved for those patients who have the neurological symptoms or questionably widespread disease. The review of bone scans in head and neck, pelvic, sarcoma and melanoma patients is still underway, but the preliminary data suggest it is not completely necessary at the screening exam.

Review of 108 consecutive diagnostic scalene lymph node biopsies revealed 12 patients with metastatic tumor to the prescalene nodes. In six of the 12 positive biopsies, the nodes were clinically suspicious. Six patients with clinically negative scalene nodes showed microscopic occult metastases; five of these patients had squamous carcinomas of the pelvis. The overall occult metastatic detection rate of 6% suggested limited usefulness of blind scalene node biopsy as a screening test.

Results of the reviews of excretory urography and gastrointestinal series as screening measures for occult metastatic tumor are presently being evaluated.

Significance to Cancer Research and the Program of the Institute:

The assessment of the sensitivities and specificities of various screening tests has obvious importance in cancer patients for the detection of extent of disease.

The review of the Surgery Branch experience indicates that certain screening tests are particularly valuable in the detection of metastatic disease, while others appear to be of limited usefulness. The sensitivities of each test depended to some degree on the type of malignancy. Chest tomography appeared to be an extremely sensitive method for demonstrating pulmonary metastases in all types of cancers. Liver scans were of value in screening patients with melanoma and with pelvic neoplasms, both diseases with a reasonably high rate of hepatic metastases. However, liver scans were not of value in sarcomas or in head and neck cancers which only rarely spread to the liver. Brain scans were considered to be useless in the screening of neurologically asymptomatic patients. Scalene node biopsies appeared to be possibly useful only in patients with advanced pelvic neoplasms. The relative values of other tests is presently under evaluation.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

The results of the tests reviewed thus far appear to define the most useful screening measures for the following malignant diseases: sarcomas, melanoma, head and neck lesions, and advanced pelvic tumors. The value of these tests in other malignancies, such as breast cancer or colorectal carcinomas, has not been determined because of insufficient numbers of such patients. Further efforts should be made to specify the value of the various screening measures in these common types of tumors.

Keyword Descriptors:

Tomography, nuclear scans, screening.

X. Project Description: Computer Applications to Research and Clinical Problems in the Surgery Branch

Part a. Serum Bank

Objectives:

The objective of this project is to design a system to catalogue and store serum that is well characterized clinically, is rapidly accessible, and updatable.

Methods Employed:

The Surgery Branch in collaboration with the Division of Computer Research Technology has designed a system with the following specifications:

(1) Design storage programs for:
  (a) Biographical patient information used to identify the individuals (name, age, sex, etc.).
  (b) Coded (Manual of Tumor Nomenclature and Coding (MOTNAC)) description of each patient's primary tumor by site, histology, and extent.
  (c) Serum identification number with corresponding patient identification number, date of acquisition, and the quantity of serum on hand.
  (d) Experimental data generated from specific serum samples.

(2) Design retrieval programs to identify records that meet selection criteria, example site and histology, and produce data output formatted in a usable form.

A system meeting these specifications was developed using standard data processing methods.

Major Findings:

A system that will perform the above described functions has been operational since September 1974. More than 4,000 separate individual patient records have been incorporated into the data base and 15,000 separate serum drawings have been catalogued and stored frozen in liquid nitrogen.

The banking and retrieval system has been used in the following projects to identify appropriate serum specimens for sampling:

(1) Study of nonvirion antigen in head and neck cancer patients.
(2) Study of IgG, IgA, and IgM viral antibodies in the serum of head and neck cancer patients, normal nonsmoking controls, and normal controls who smoke.
(3) Study of glycoprotein carbohydrate patterns of tumor-bearing and cured head and neck cancer patients.
(4) Study of specific glycoprotein changes in malignancy by immunodiffusion techniques.

(5) Study of serum antibodies to nonviral antigens by herpesvirus in patients with bladder cancer.
(6) Study of carcinoembryonic antigen in head and neck cancer patients.
(7) Study of the presence and disappearance kinetics of CEA in nontumor-bearing smokers.
(8) Study of CEA in patients with the following malignancies:
  (a) Esophagus
  (b) Melanoma
  (c) Sarcoma
  (d) Lung cancer
(9) Study of colon membrane associated tumor-specific antigen in adenocarcinomas of the colon.

Significance to Cancer Research and the Program of the Institute:

The development of a bank with the described capabilities is of central importance in large-scale serologic studies of cancer patients.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

The projected applications of the serum bank storage and retrieval system are in principle limited only by the types of individuals who are incorporated. Projected applications at the present time include the following:

(1) Study of glycoprotein changes in smokers.
(2) Study of glycoprotein changes in cancer patients undergoing radiation therapy.
(3) Study the effect of serum from cancer patients with known glycoprotein carbohydrate concentrations on normal lymphocyte reactivity to phytohemagglutinin.

Part b. Revision of Clinical Protocols

Objectives:

The Surgery branch has been involved in the accumulation of large volumes of clinical information on patients seen and treated by our unit for approximately 4 years. With recognition of the changing needs of the Surgery Branch, we have undertaken a major revision in the data to be stored and its use. The objectives of the redesigned system are:

(1) Storage of minimal but extremely accurate information on each patient.
(2) Storage of an updated assessment of the patient's tumor status.
(3) Routine production of computer-generated summaries of the data input for each patient so that it is available and helpful in the ongoing follow-up of each patient.
(4) Develop standard retrieval programs for the generation of survival rates, recurrence rates, disease-free intervals, and statistics

describing the characteristics of our patient population.

(5) Develop retrieval programs to identify individual patients that meet specific selection criteria (i.e., females between ages 30 and 50, operated on for adenocarcinoma of the breast greater than 2 years prior to the date of origination of the request).

Methods Employed:

Standard computer programming techniques will be employed to generate the storage and retrieval programs necessary to meet the described criteria. This work will be done in conjunction with the Divison of Computer Research Technology at the National Institutes of Health.

Major Findings:

This project is in its developmental stages at the present time. The projected date of June 1 is given for initial operational status of this project.

Significance to Cancer Research and the Program of the Institute:

The necessary group of programs to accomplish the described objectives of this project will enable the Surgery Branch to maintain an up-to-date and useful record of the individuals we are following in a rapidly accessible and useful form. The data accumulated by this project can be used to generate many useful statistics concerning recurrence and survival in the individual diseases that we are involved in treating. This project will also provide the individual physicians who are involved in the daily care of Surgery Branch patients with an up-to-date computer-generated synopsis of the individual, his diagnosis, bibliographic data, and the history of his disease in terms of tumor status.

NCP Objectives and Approaches: 04, A1,2,3; 05, A4,6; 06, A1,2,3; 07, A2

Proposed Course:

This project will be taken to completion, at that time it will be incorporated in the clinical program of the Surgery Branch and will be used to catalogue and record clinical data generated on the patients who are seen and treated by our unit.

Keyword Descriptors:

Computers, serum bank, clinical data retrieval.

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Serial No. Z01-CBD-3807-01-SURG

1. Surgery Branch
2. Tumor Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunoevaluation of Patients with Cancer

Previous Serial Number: None

Principal Investigator: P. B. Chretien, M.D.,

Other Investigators: N. Silverman, M.D., J. Alexander, M.D., H. Smith, M.D. and C. Potvin, M.D.

Cooperative Units: None

Man Years:

Total: 4
Professional: 2
Other: 2

Project Description:

Part I; The Effect of Nonthymic Irradiation on Cellular Immunocompetence.

Part II: The Effect of Chronic Cigarette Consumption on Systemic Cellular Immunocompetence

Part III: Immune and Virological Abnormalities in Squamous Head and Neck Carcinoma; Relationship to Tumor Burden and Site

Part IV: Study of Humoral Immunity to Herpesvirus-Induced Antigens in Cancer Patients

Keyword Descriptors: Immunocompetence, cigarette smoking, herpesvirus antigens, T-cells, B-cells, PHA stimulation, lymphocytes

Project Description:

Part I: The Effect of Nonthymic Irradiation on Cellular Immunocompetence

Objectives:

Radiation therapy delivered by mediastinal portals has been shown to cause depression of cellular immunity acutely, and this suppression persists for at least 2 years. The contributing roles of direct thymic injury and injury to lymphocytes circulating through the radiation portals have not been elucidated. To delineate the mechanism, extent, and duration of the deleterious effect of irradiation on immune reactivity, lymphocyte reactivity (LR) to phytohemagglutin (PHA) and peripheral blood lymphocyte subpopulations were determined in patients with squamous carcinoma of the head and neck region studied at intervals during and after radiation therapy.

Methods Employed:

Lymphocytes were separated on a Ficoll-Hypaque gradient. The percentage of T cells were determined by the formation of nonimmune rosettes with sheep erythrocytes. By convention, lymphocytes with three or more adherent sheep erythrocytes were considered T cells. B lymphocytes were detected by the percentage of lymphocyte-forming rosettes with erythrocytes coated with 19S hemolysin and mouse complement. All samples were done in triplicate. Lymphocyte reactivity (LR) to phytohemagglutin (PHA) was determined in microtitre plates. Lymphocytes were incubated with 20% AB serum, RPMI 1640 media, and 4 concentrations of PHA for 72 hours. Tritiated thymidine was added 6 hours prior to termination of the culture. Cells were harvested and counted in a Packard Tri-Carb Scintillation Counter and the absolute activity was expressed in DPMs. The LR was the mean of the highest triplicate DPM's minus the DPM of control cultures without mitogen.

Major Findings:

During radiation therapy, LR to PHA was significantly depressed after 2,000 rads tumor dose and fell progressively to 40% of pretreatment levels after 6,000 rads. Both percentage and absolute T cell levels declined similarly. Percentage B cell levels rose reciprocally but absolute B cell levels were unaltered. Depression of cellular immunity was similar in patients who received irradiation to the primary only and those treated via portals that also included the neck region. The changes were not related to the therapeutic effect of the irradiation.

After completion of radiation therapy, LR did not rise until greater than 2 months and did not return to pretreatment levels until 3-4 years. T cell levels did not rise until greater than 2 years and reached pretreatment levels only after 5 years. In patients with tumors persisting after irradiation, LR and T cell levels did not rise after completion of therapy.

Significance to Cancer Research and Program of the Institute:

The data show that irradiation via portals that do not encompass the thymus or large areas of bone marrow greatly suppresses cellular immune reactivity for prolonged intervals. The depression is due to the selective deletion of the T cell population from the peripheral blood. The findings may be related to the failure of cure rates to be substantially improved in several clinical trials with radiation therapy used as an adjunct to surgery in the treatment of resectable malignancies.

NCP Objectives and Approaches: 04, A1; 05, A1, 2, 3, 4, 5; 06, A1, A4

Proposed Course:

The role of serum suppressant factors in the immune suppression induced by irradiation will be investigated. Lymphocytes from irradiated patients will be washed repeatedly and the LR of these cells will be compared to the LR of lymphocytes processed by usual methods. Serum glycoprotein abnormalities will be correlated with the immune defects occurring during irradiation. The potential suppressive effect of serum from irradiated patients on normal lymphocytes will also be evaluated.

Project Description:

Part II. The Effect of Chronic Cigarette Consumption on Systemic Cellular Immunocompetence

Objectives:

It has been suggested that suppression of immune responses by tobacco products may be an important event in the genesis of malignancies associated with cigarette consumption. This speculation derived primarily from evidence that tobacco products, in addition to being carcinogenic, are also immunosuppressive and demonstrations of an increased incidence of neoplasia in immune-suppressed hosts. Hypotheses concerning the relation between tobacco consumption, immune suppression and malignancy give importance to a determination of the effects of chronic cigarette consumption on cellular immunity in humans. These effects are assessed by comparing the reactivity *in vitro* to PHA of peripheral blood lymphocytes and the peripheral blood levels of thymus-dependent lymphocytes (T cells) in smoking and non-smoking normal subjects.

Methods Employed:

Healthy volunteers who had never consumed or who consumed cigarettes on a daily basis were tested. Individuals were grouped by age and smoking history (pack-years). Total blood leukocytes were determined on a counter particle counter. Leukocyte differential counts were determined in Wright stained smears of peripheral blood. Lymphocytes were separated on a Ficoll-Hypaque gradient. The percentage of T cells and lymphocyte reactivity to PHA were determined as described in Part I.

Major Findings:

The mean total blood leukocytes/mm^3 (WBC) of all smokers was significantly higher than that of the nonsmokers. There was a significant increase in WBC with age in the smokers, but not the nonsmokers. The mean WBC of smokers in the 4th, 5th and 6th decades was higher than the nonsmokers. The absolute lymphocytes/mm^3 of the smokers was significantly higher than nonsmokers, irregardless of age or pack-years. The percentage T cells in each group were similar. However, the absolute T cells/mm^3 were significantly elevated in the smoking population. There was no correlation between absolute T cells and age or pack-years. The mean LR of the smokers was greater than nonsmokers. This occurred in smokers less than forty years of age, or who had a 20 or less pack-year history of cigarette consumption. Older smokers or those with heavier smoking histories had similar LR compared to nonsmokers.

Significance to Cancer Research and Program of the Institute:

The data indicates that chronic cigarette consumption does not suppress systemic cellular immunocompetence, but stimulates immune reactivity as measured by these in vitro parameters.

NCP Objectives and Approaches: 04, A1; 05, A1, 2, 3, 4, 5; 06, A1, 4

Proposed Course:

Studies are in progress to determine the reversibility of these immune changes after the cessation of cigarette smoking. The immune reactivity and T cell levels will also be correlated with CEA levels and glycoprotein abnormalities seen in the blood of the smoking population.

Project Description:

Part III. Immune and Virological Abnormalities in Squamous Head and Neck Carcinoma; Relationship to Tumor Burden and Site

Objectives:

Defects in cellular immune competence have been found in localized, solid malignancies. Patients with squamous cell carcinoma of the head and neck region differ from patients with other histologic types of malignancies in that the preoperative immune defect persists in cured patients. Recent studies have shown a high incidence of antibodies to herpes virus (HSV) - induced nonvirion antigen (NVA) in the serum of patients with squamous cancer of the head and neck region and a similar high incidence in cured patients. Because of the parallelism between persisting cellular immune defects and antibodies to HSV-NVA in patients cured of head and neck squamous carcinomas, the present study was conducted to further define the unique defects in cellular immunity associated with these tumors and to correlate the defects with the incidence of antibodies to HSV-NVA.

Methods Employed:

Healthy volunteers and patients with biopsy proven squamous cell carcinoma of the head and neck region were studied. Lymphocytes were separated on a Ficoll-Hypaque gradient. Lymphocyte reactivity (LR) to phytohemagglutin (PHA) was determined in microtitre plates as described in Part I. NVA was prepared from cultured human fetal kidney cells infected with HSV. Cells were harvested at 24 hours, sonicated, and centrifuged. Pellets were resonicated and centrifuged. The supernatants were pooled, concentrated, and separated by PAGE. Region 3 of each gel was sliced and the protein eluted. Standard complement-fixation tests were performed with cancer and control serum.

Major Findings:

The mean LR of all tumor-bearing (TB) and cured patients was depressed compared to controls. In the cured patients, those who had received prior irradiation had similar LR as those who had had surgery alone as their treatment modality. There was no difference in LR between TB and cured patients. The patients were then grouped by anatomical site, clinical stage, and tumor status. The mean LR of all patients with oral cavity and oropharyngeal tumors and those with Stages II-IV laryngeal tumors were depressed compared to normals. Those patients with tumors confined to the vocal cord were unique in manifesting normal immunocompetence. This was true in both TB and cured patients. Antibodies to HSV-NVA were found in 89% of patients and 0% of controls. The incidence was similar in cured and TB patients. Patients with Stage I laryngeal tumors were again unique in having a lower incidence of antibodies to HSV-NVA than patients with more advanced laryngeal tumors and tumors at other sites. However, these Stage I laryngeal patients had a higher incidence than the controls.

Significance to Cancer Research and Program of the Institute:

This data indicates that impaired LR in patients with squamous carcinoma of the head and neck is not solely due to the persistent immunosuppressive effects of prior tumor treatment (i.e. irradiation) and that the immune defect appears related to tumor burden prior to definitive therapy. The incidence of antibodies to HSV-NVA in the serum of these patients parallels the occurrence of persistent defects in cellular immunity. In view of the demonstrated suppression of cellular immunity by viral infection, a similar association between the production of HSV-NVA and systemic cellular immune defects may be postulated.

NCP Objectives and Approach: 04,A1; 05,A1,2,3,4,5; 06,A1,A4

Proposed Course:

The development of a radioimmunoassay for HSV-NVA will allow more sensitive detection and localization of this neoantigen. Individuals at high risk of developing head and neck cancer, i.e. heavy smokers, will then be screened to see if HSV-NVA can be detected prior to the development of overt malignancy.

Project Description:

Part IV. Study of Humoral Immunity to Herpesvirus-Induced Antigens in Cancer Patients

Objectives:

Epidemiologic and serologic studies have associated the herpes simplex viruses with squamous carcinomas of the cervix and head and neck. To date there is no convincing evidence for a viral etiologic role, but such studies have suggested that herpesvirus-induced antigens are present in some human tumors and are recognized as foreign by the host's humoral immune system. This study was undertaken to more clearly define the nature of the humoral immune responses to herpesvirus-induced antigens in patients with squamous carcinomas and appropriate control populations.

Methods Employed:

An indirect immunofluorescent assay was developed to detect human antibodies directed against viral-induced antigens. Monkey kidney cells, infected with higher titer herpes simplex virus Type I, were harvested from monolayer cultures and used, in suspension, as target cells. Goat anti-human IgG, IgA, and IgM (heavy chain-specific) antisera, conjugated with fluorescein isothiocyanate, were obtained commercially. Their monospecificity was confirmed using purified immunoglobulin heavy and light chain moieties bound to activated Sepharose beads. The assay was semi-automated so that 128 sera could be simultaneously studied to determine relative levels of antibody against viral-induced antigens by an endpoint titration method.

Major Findings:

1. Serum IgA antibodies against herpes simplex viral-induced antigens (Ig anti-HSVIA) were detected in significantly greater frequency of patients with squamous carcinomas of the head and neck, squamous carcinomas of the cervix, and squamous carcinomas of the skin than in controls with nonsquamous carcinomas or no smoking history. There were no significant differences in the frequencies of IgG or IgM anti-HSVIA antibodies among the groups studied.

2. In patients tumor-free after excision of squamous carcinomas of the cervix, the frequency of IgA anti-HSVIA was lower than in the group of tumor-bearing patients but higher than in controls.

3. In patients tumor-free after excision of squamous carcinomas of the head and neck, the frequency of IgA anti-HSVIA was comparable to that of tumor-bearing patients.

4. A group of individuals matched with squamous carcinoma of the head and neck patients for age, sex, and smoking history had a frequency of IgA anti-HSVIA greater than in nonsmokers and nonsquamous tumor-bearing patients but similar to that in tumor-bearing and tumor-free squamous carcinoma of the head and neck patients.

5. A group of heavy smokers matched for age and sex with the tumor-bearing squamous carcinoma of the cervix patients had a frequency of IgA antiherpes simplex viral-induced antigen antibodies significantly lower than tumor-bearing squamous carcinoma of the cervix patients but not statistically different from tumor-free patients.

Significance to Cancer Research and the Program of the Institute:

The production of IgA antibodies to herpesvirus-induced antigens in patients with squamous carcinomas indicates that activation of latent herpesviruses with production of viral-induced antigens may accompany dysplastic or neoplastic transformations of mucosal cells. Such virus-induced antigens could prove to be valuable markers for the presence of altered tissue as well as targets for immunotherapeutic manipulations.

In this study, excision of squamous carcinomas of the cervix significantly reduced the antibody production in the group of patients as a whole as well as in individual patients studied longitudinally. This observation suggests that such an assay may be useful in monitoring patients for residual or recurrent malignant disease.

In contrast, excision of squamous carcinomas of the head and neck did not reduce the antibody production. Such tumors usually arise in mucosa characterized by generalized dysplasia and multiple sites of in-situ anaplasia. Tumor resection removed only a fraction of this altered mucosa which may itself have been a source of viral-induced antigens. This latter possibility is supported by the fact that heavy tobacco consumption, known to produce dysplasia of the nasopharyngeal mucosa, was associated with stimulation of serum IgA anti-HSVIA antibodies. This observation also indicates that an important action of carcinogenic agents, such as tobacco products, may be the activation of viruses, leading to viral gene product synthesis in tissue destined to become dysplastic or malignant.

NCP Objectives and Approach: 04, A1; 05, A1, 2, 3, 4, 5; 06, A1, A4

Proposed Course:

(1) More patients with squamous carcinomas will be studied longitudinally to determine the true value of this assay in monitoring for residual or recurrent squamous cancer. (2) The population of heavy smokers studied will be followed to evaluate the ability of this assay to predict the development of head and neck squamous carcinomas in a population at risk.

Publications:

Catalona, W.J., Tarpley, J.L., Potvin, C., and Chretien, P.B.: Correlations among cutaneous reactivity to DNCB. PHA-induced lymphocyte blastogenesis and peripheral blood E Rosettes. Clin. Exp. Immunol. 19:327-333, 1975.

Catalona, W.J., Potvin, C., and Chretien, P.B.: T lymphocytes in bladder and prostatic cancer patients. J. Urol. 112:378-382, 1974.

Catalona, W.J., Potvin, C., and Chretien, P.B.: Effect of radiation therapy for urologic cancer on circulating thymus-derived lymphocytes. J. Urol. 112: 261-267, 1974.

Potvin, Claude, Dellon, A. Lee and Chretien, Paul B.: T-cell levels in bronchogenic carcinoma: Correlations with tumor stage and clinical course. Surgical Forum, SSV, 96-97, 1974.

Tarpley, John L., and Chretien, Paul B.: Prolonged depression of cellular immunity in cured laryngopharyngeal cancer patients treated with radiation therapy. Cancer 35:638-644, 1975.

Dellon, A. Lee, Potvin, Claude, and Chretien, Paul B.: Thymus-dependent lymphocyte levels in bronchogenic carcinoma: Correlations with histology, clinical stage, and clinical course after surgical treatment. Cancer 35: 687-694, 1975.

In Press:

Dellon, A. Lee and Chretien, Paul B.: Thymus-dependent lymphocyte levels during radiation therapy for bronchogenic and esophageal carcinoma: Correlations with clinical course in responders and nonresponders. The Am. Jour. Roent., Radium Therapy & Nuclear Med.

Potvin, Claude, Tarpley, J.L., and Chretien, Paul B.: Thymus-derived lymphocytes in patients with solid malignancies. Clin. Immuno. & Immunopath.

Tarpley, J.L., Catalona, W.J., and Chretien, P.B.: Effect of previous irradiation on DNCB reactivity in cancer patients. Surgery.

Serial No. Z01-CBD-3809-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In Vitro Responsiveness to Thymosin of Lymphocytes from Patients with Solid Malignancies

Previous Serial Number: None

Principal Investigator: Paul B. Chretien, M.D.

Other Investigators: Claude Potvin, M.D. and Daniel Kenady, M.D.

Cooperating Units: None

Man Years:

Total: 3
Professional: 1-1/2
Other: 1-1/2

Keyword Descriptors: Thymosin, T-cells, Immunodepression

Project Description:

Objectives:

In studies conducted with lymphocytes from children with thymic deficiency syndromes, an increase in the number of lymphocytes forming spontaneous rosettes with sheep red blood cells occurs with the addition of thymosin. After subsequent administration of thymosin *in vivo* to these patients, this increase in the percentage of lymphocytes forming spontaneous rosettes (the thymus-dependent lymphocytes or T cells) no longer occurs. This correlation suggests that an increased percentage of T cells *in vitro* after the addition of thymosin, using the spontaneous rosette technique, may be indicative of *in vivo* thymosin deficiency. In an attempt to define patient populations with solid malignancies that may display significant thymosin deficiency by this assay technique, the relative responsiveness to thymosin *in vitro* of lymphocytes from patients with a variety of solid malignancies and a subset of patients undergoing irradiation to the mediastinum or pelvis were studied. Lymphocytes from healthy volunteers were studied simultaneously with the cancer patients.

Methods Employed:

Thus far, lymphocytes from 67 normal volunteers, over 100 patients with solid malignancies studied prior to treatment, and over 200 patients with solid malignancies studied during irradiation of the mediastinum or pelvis have been assayed. T cell levels were measured as described previously.

Major Findings:

As in previous studies of patients with solid malignancies, the T cell levels of the patients in this study were, as a group, lower than that of normals. During the course of irradiation to either the mediastinum or pelvis, T cells dropped to a mean of less than 50% of pretreatment levels. Significant changes in non-T cell levels, i.e., a combination of B cells and "null" cells, did not occur during the course of irradiation. This data parallels that observed in a detailed study of T and B cell levels during irradiation for primary carcinomas of the head and neck region. Preliminary analysis of the response *in vitro* to the addition of thymosin shows a relation between the control T cell levels and the increase in T cell levels after thymosin. The greatest response to *in vitro* thymosin, both in the percentage of samples displaying a rise in T cells and in the magnitude of the rise occurred in patients with extremely low control T cell levels. Further analysis of this data and the study of additional patient populations will be required to substantiate this tentative conclusion and to allow correlations with tumor histology, amount of irradiation administered, and the effects of individual radiation portals on the *in vitro* responsiveness of lymphocytes to thymosin.

Significance to Cancer Research and Program of the Institute:

The demonstrated efficacy of thymosin in reconstituting cellular immunity in children with thymic deficiency syndromes suggests a use for this agent in the treatment of cancer patients. In defining patient populations that may benefit from adjuvant therapy with thymosin, it is relevant that the resting T cell levels of patients with potentially curable malignancies does not differ greatly from that of normals, although the mean differences are statistically significant. On the other hand, all currently used therapeutic modalities, i.e., surgery, radiation therapy, and chemotherapy lower T cell levels for variable periods of time. It may be hypothesized that this effect on T cell levels may have a deleterious effect, particularly by facilitating the survival of circulating tumor cells and propagation of these cells at other sites. As a preliminary step in developing a rationale for the adjuvant use of thymosin to counteract the T cell suppressive effect of cancer therapy, the responsiveness to thymosin of the residual circulating lymphocyte fraction must be determined. The goal of this study is to determine whether the circulating lymphocytes remaining after radiation therapy respond *in vitro* to thymosin by an elevation in the percentage of T cells. This determination is the goal of the present study. If positive results are obtained, an in vivo determination of the effects on T cell levels and in vivo cellular immune competence in patients undergoing radiation therapy would appear warranted.

NCP Objectives and Approach: 04, A1; 06, A1,4

Proposed Course:

If the present study demonstrates that a significant fraction of the residual circulating lymphocytes respond to thymosin in vitro by an increase in the percentage of T cells, a protocol for a determination of the effects of thymosin in vivo will be formulated that would be conducted in patients with inoperable carcinoma of the lung treated by radiation therapy. Similar studies will be conducted with lymphocytes from patients undergoing chemotherapy with the goal of determining by in vitro assays whether a rational basis for a trial of thymosin as an adjuvant to chemotherapy can be developed.

Publications:

None

Serial No. Z01-CBD-3810-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Circulating Glycoproteins of Cancer Patients

Previous Serial Number: None

Principal Investigators: C.B. Cuono, M.D., Ph.D., J.C. Alexander, M.D., P.B. Chretien, M.D., R.M. Beazley, M.D.

Other Investigators: C. Gorschboth, B.S.

Cooperating Units: None

Man Years:

Total:	2-1/2
Professional:	1-1/2
Other:	1

Project Description:

Part I.	Development of New Techniques for the Separation of the Components of Human Serum Seromucoid
Part II.	Development of Automated Techniques for the Measurement of Serum Carbohydrates
Part III.	Carbohydrate Abnormalities in Cancer Patients
Part IV.	Glycoprotein Abnormalities in Cancer Patients

Keyword Descriptors: Seromucoid, glycoproteins, isoelectric focusing

Project Description:

Part I. Development of New Techniques for the Separation of the Components of Human Serum Seromucoid

Objectives:

The development, spread, or recurrence of malignancy is often accompanied by elevations in the seromucoid fraction of serum, a heterogenous population of non-immunoglobulin glycoproteins. Immunologic evidence indicates the proteins of this class interact with tumor cell surfaces and are endowed with immunosuppressive properties. A major obstacle to their investigation has been the lack of specific methodology for separating these proteins from one another in serum. The objective of this study is to develop a method to separate the components of seromucoid, thus obtaining a glycoprotein profile. The study would then be extended to assess the possibility that such glycoprotein profiles in malignant disease may be unique.

Methods Employed:

Seromucoid is obtained by precipitating the non-seromucoid proteins from serum with perchloric acid. The acid supernatant containing seromucoid proteins is neutralized, de-salted and concentrated and is subjected to fractionation. None of the well established protein separation techniques offered the degree of resolution required considering the small quantity of seromucoid obtained from a reasonably sized serum sample. It was felt that isoelectric focusing in polyacrylamide gel (PAGIF), a recently developed high resolution in protein separation method, would possibly effect superior separation and resolution of the microgram quantities of seromucoid available from a given sample. In PAGIF proteins are separated according to their isoelectric points in an electrically established continuous pH gradient. The isoelectric point of a protein is characteristic. Furthermore, normal or abnormal variants of a given protein exhibit differing isoelectric points, though the magnitude of the difference may be small. However, by choosing an appropriately narrow pH range and experimental conditions, protein species whose isoelectric points differ by as little as 0.005 pH unit can be resolved by PAGIF.

Major Findings:

Initial studies with a steep pH gradient of 2-11 revealed all serum proteins to focus at a pH below 7. Utilizing a narrower pH gradient with a nominal range of 2.5-7, approximately 20 components were visualized. The broad band at the anodal end of the gel was seen to consist of several tightly spaced bands which were thought to represent variants of a single protein. By focusing the same mixture over a still narrower pH gradient of 2.5-4, these particular bands were effectively separated from one another. Using crossed immunoelectrophoresis, these particular bands were unequivocally identified as the variants of α_1-acid glycoprotein, a protein known to be elevated in many malignant diseases. Using similar immunochemical techniques, the presence and position of α_1-antitrypsin has been identified.

Significance to Cancer Research and Program of the Institute:

Elevations in serum glycoproteins are not unique to the malignant state. A variety of pathologic conditions are associated with increased whole seromucoid levels. In view of this apparent lack of specificity, earlier investigators maintain that seromucoid determinations were of little value as diagnostic indicators in patients with cancer, though serial seromucoid determinations have been shown to be quite reliable prognostic indicators in cancer patients. More recent study of several of the known components of seromucoid has demonstrated elevations in some of the components and decreases in others in a population of cancer patients without parallel changes in non-malignant disease controls. Clearly, the pattern of glycoprotein alterations in seromucoid of cancer patients is considerably more complex than originally considered, and still largely obscure.

The development of this facile technique for separation of these components of seromucoid effectively removes a major obstacle to their in-depth investigation in malignant disease.

NCP Objectives and Approach: 04, A1

Proposed Course:

In ongoing studies in the Surgery Branch, efforts are currently being directed at the identification of the remaining bands presently in normal seromucoid. Plans are to systematically study homogeneous subsets of patients with malignant disease and non-malignant diseases hopefully to discern the presence of profiles which may be characteristic of or even pathognomonic for specific pathologic entities.

Project Description:

Part II. Development of Automated Techniques for the Measurement of Serum Carbohydrates

Objectives:

To develop efficient reliable automated assay procedures for hexoses, hexosamines, fucose and sialic acid present in serum glycoproteins and to study differences in carbohydrate composition of serum glycoproteins in cancer patients.

Methods Employed:

An automated technique is currently under development for the chromatographic (ion-exchange) separation of individual hexoses and hexosamines from protein hydrolysates. These will be quantitated spectrophotometrically. An automated assay has been developed for determination for serum fucose via colorimetric means. An automated procedure is under early development for assay of free and glycosidically-bound sialic acid.

Major Findings:

Efforts thus far have demonstrated the feasibility of rapid automated fucose determinations. Assays for differential determination of hexoses and hexosamines are still under development as is the sialic acid assay. No major findings with respect to cancer patients can as yet be reported.

Significance to Cancer Research and Program of the Institute:

It is anticipated that data on groups of cancer patients will bring to light aberrations in the carbohydrate composition of the serum glycoproteins as has been shown in various animal models. Knowledge of these carbohydrate aberrations will be significant in the overall study of the immunosuppressive properties of these proteins with respect to neoplasia.

NCP Objectives and Approach: 05, A4

Proposed Course:

The assays as currently being developed are to be applied to the study of groups of patients with malignant disease in order to assess the possibility of specific serum carbohydrate abnormalities in these patients as compared with appropriate controls.

Project Description:

Part III. Carbohydrate Abnormalities in Cancer Patients

Objectives:

The objective of this phase of the study is to ascertain the changes in the individual carbohydrate components of glycoprotein; sialic acid, neutral hexose, hexosamine, and fucose and correlate these values with tumor histology, tumor burden and lymphocyte reactivity to phytohemagglutinin.

Methods Employed:

Lymphocyte reactivity is determined by the microtiter lymphocyte culture technique using phytohemagglutinin as a mitogen. Tumor burden was estimated by analysis of the patient's clinical chart; histology of the individual tumors was determined by hematoxylin-eosin stain examination of appropriate biopsies. Total protein was determined by the Biuret method; sialic acid was determined by the Warren thiobarbituric acid method; the hexoses were determined by color reactions; fucose was determined by a modification of the Dische-Shettle cysteine-sulfuric acid reaction.

Major Findings:

Sialic acid, neutral hexose, hexosamine, and fucose were all found to be elevated in the malignant state. The degree of elevation was related to tumor burden, i.e., a small tumor burden gave small changes in the mentioned compounds; however, a large tumor bruden gave large alterations in the compounds measured. Sera was examined from individuals bearing squamous cell tumors of the head and neck, pelvis, adenocarcinomas, sarcomas, and melanomas. There was no difference found in the abnormalities between histologic types in the tumor-bearing state. When individual sera were examined from patients cured of the above malignancies, all of the separate histologic types except squamous cell carcinoma reverted to normal. Sialic acid, neutral hexose, hexosamine, fucose, all remained elevated in patients cured of squamous cell carcinoma. The elevation was not as great as the tumor-bearing state; however, it was still significantly above normal. Lymphocyte reactivity in these same groups was examined and inversely correlated with the degree of abnormality in the glycoprotein carbohydrates. Lymphocyte reactivity became increasingly depressed with increasing tumor burden. In all histologies except squamous cell carcinoma, lymphocyte reactivity in individuals cured of their tumor was normal. The lymphocyte reactivity in squamous cell carcinoma patients who have been cured of their disease for greater than two years remains depressed.

Significance to Cancer Research and Program of the Institute:

The assays of sialic acid, neutral hexose, hexosamine, and fucose may be useful as an estimate of tumor bruden in patients diagnosed as having malignancies of any histologic type. The determination could not be used as a histologically specific tumor marker. The relationship of the abnormalities in glycoproteins to lymphocyte reactivity may suggest a regulatory role for glycoproteins in the patients studied. Carbohydrates in general and glycoproteins specifically may be involved in regulation of lymphocyte reactivity.

NCP Objectives and Approach: 05, A6

Proposed Course:

The assay methods described and their relationships will need to be examined in greater detail in these and other tumor systems. The relationship of glycoprotein abnormalities to lymphocyte reactivity needs to be examined in greater depth.

Project Description:

Part IV. Glycoprotein Abnormalities in Cancer Patients

Objectives:

The concentration of specific glycoprotein species will be correlated with tumor histology, tumor burden, and lymphocyte reactivity in patients with various histologic types of malignancy, both tumor-bearing and cured, and controls.

Methods Employed:

Lymphocyte reactivity is determined by the microtiter lymphocyte culture technique using phytohemagglutinin as the stimulating mitogen. Tumor burden and histologic type were determined as in Part III of this study. The specific glycoprotein moieties, acid glycoprotein, alpha I antitrypsin, ceruloplasmin, haptoglobin, HS glycoprotein, alpha II macroglobulin, alpha II HS glycoprotein, beta lipoprotein, transcortin, hemopexin, beta II glycoprotein, beta IE and beta IC, (complement components), IgG, IgA, IgM, and plasminogen were measured by radial-immunodiffusion techniques.

Major Findings:

This work is in its initial stages and there are no major findings as yet.

Significance to Cancer Research and Program of the Institute:

The questions to be examined when the data is analyzed are whether or not a specific glycoprotein can be used to denote the presence of a specific histologic type of tumor and/or the volume of tumor present. The relationships between lymphocyte reactivity and specific glycoprotein moieties will be examined to determine whether there is any one segment of the glycoprotein fraction that correlates either in a direct or inverse fashion with lymphocyte reactivity.

NCP Objectives and Approach: 05, A4

Proposed Course:

The extension of this will depend upon the analysis of the data.

Publications:

None

Serial No. Z01-CBD-3811-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Immunotherapy of Animal and Human Sarcomas

Previous Serial Number: None

Principal Investigator: Steven A. Rosenberg, M.D., Ph.D.

Other Investigators: G.A. Parker, M.D., G.S. Leight, M.D., S. Schwarz

Cooperating Units: None

Man Years:

Total:	3-1/2
Professional:	1-1/2
Other:	2

Keyword Descriptors: Sarcomas, immunotherapy, immunologic assays

Project Description:

Objectives:

To study the humoral and cellular responses of the host to growing sarcomas. To evaluate the effects of therapy on the immune response to these sarcomas. To develop methods of immune manipulation to affect the growth of sarcomas in animal and human hosts.

Methods Employed:

This project was begun in the last few months of the period covered by this annual report. Assays are being developed to monitor the humoral and cellular immune responses to humans with soft tissue sarcomas and animals with methylcholanthrene induced sarcomas. The major humoral assays being developed are the C1 fixation and transfer test, complement mediated cytotoxicity, and a radioactive isotope hemadsorption assay. The primary cellular immune assays being developed involve the use of cytotoxicity and mixed lymphocyte tumor culture assays.

A variety of human soft tissue and bony sarcomas and mouse methylcholanthrene induced sarcomas have been put into long term tissue culture. Where possible corresponding normal tissue has also been cultured.

Major Findings:

This project has just begun and there are no major findings as yet.

Significance to Cancer Research and Program of the Institute:

Soft tissue and bony sarcomas in humans have an overall five year survival of about 30-40%. Our studies in the laboratory are aimed at developing immunotherapeutic methods for improving the treatment of patients with these soft tissue sarcomas.

NCP Objectives and Approach: 06, A1,2,4

Proposed Course:

This study has just begun and will be continued during the next year.

Publications:

None

Serial No. Z01-CBD-3812-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies with a Chemically-induced Rat Colon Cancer Model

Previous Serial Number: None

Principal Investigator: Alan R. Baker, M.D.

Other Investigators: J. C. Marrone, A.B.

Cooperating Units: None

Man Years:

Total:	2
Professional:	1
Other:	1

Keyword Descriptors: Rat, colorectal cancer, dimethylhydrazine, N-methyl, N-nitro, N-nitrosoguanadine, TSTA, CEA.

Project Description:

Objectives:

In the past few years several chemical carcinogens have been described which exhibit a high degree of colonotropism in the rat. The objective of this study is to explore the potential of this system as a model for human colorectal cancer. Efforts will be made to induce tumors with dimethylhydrazine and N-methyl, N-nitro, N-nitrosoguanadine in the rat and to define their immunogenicity both from a tumor specific and oncofetal point of view. An attempt to measure CEA-like material elaborated by the tumors is planned. The potential for studying early events associated with in vivo neoplastic transformation will be examined. Study of the utility of the model in an immunoprophylactic or immunotherapeutic context is contemplated.

Methods Employed:

A variety of in vivo and in vitro immunologic techniques will be employed. Tumors will be placed in tissue culture for use as targets in microcytotoxicity tests.

A surgical model involving the exteriorization of a segment of rat colon has been developed to facilitate studies of early events associated with in vivo transformation. Immunofluorescent techniques, on touch preparations of this exteriorized colon mucosa, will be used to correlate the appearance of de novo tumor specific or fetal specific determinants with macroscopic, cytologic, histologic, or radioautographic abnormalities.

Major Findings:

These studies are newly initiated and there are as yet no major findings.

Significance to Cancer Research and Program of the Institute:

Colorectal cancer is a major clinical problem in the country today. It has only been minimally studied in an animal model system. The recent availability of a rat colon cancer model should now permit us to ask a variety of pertinent clinical questions with the hope of obtaining answers relevant to the human situation.

NCP Objectives and Approach: 01, A4, 02, A1, 03, A1-5, 04, A2, 06, A1, 4.

Proposed Course:

As outlined under objectives.

Publications:

None

Serial No. Z01-CBD-3813-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Study of Neuraminidase as an Immunoadjuvant in the Treatment of Established Mouse Sarcomas

Previous Serial Number: None

Principal Investigator: Alan R. Baker, M.D.

Other Investigators: R. Spence, M.D., J.C. Marrone, A.B.

Cooperating Units: None

Man Years:

Total:	3
Professional:	2
Other:	1

Keyword Descriptors: Immunotherapy, neuraminidase, murine sarcoma, humoral and cellular microcytotoxicity.

Project Description:

Objectives:

There are presently only several animal model systems in which it can be shown that manipulation of the host immune system favorably alters the course of an established tumor. Simmons has demonstrated that a vaccine can be prepared by treating a syngeneic MCA mouse sarcoma with neuraminidase which, when injected in a remote location, into mice bearing this established tumor, causes total regression of the primary sarcoma in a significant proportion of the mice. The objective of this study is to repeat this in vivo observation and using suitable in vitro microcytotoxicity tests, to elucidate the mechanism of the immunotherapeutic effect.

Methods Employed:

In vivo studies -- C_3H mice are subcutaneously inoculated with syngeneic MCA sarcoma. After the primary tumor has reached a certain size, the mice are treated with multiple injections of vaccine at a site distant from the established tumor. The course of the primary tumor is followed by serially measuring its size and following the animals for survival. Appropriate controls are included to permit assessment of the results of immunotherapy.

In vitro studies -- Humoral and cell-mediated microcytoxicity techniques are currently being adapted for use in this system. Appropriate sarcoma tissue culture target cells will be labelled with I^{125}deoxyuridine. These targets will be incubated with white cells and/or serum from untreated or vaccine treated mice with tumors in varying stages of regression or progression. Quantitative assessment of the nature and degree of immunity will thus be made.

Major Findings:

This project has only recently been started. To date we have quantitated the growth characteristics of the MC-80 sarcoma in vivo.

Significance to Cancer Research and Program of the Institute:

One of our primary objectives is to gain a fuller understanding from an immunologic perspective of the interaction between the tumor and host and to learn how to tip this delicate balance, through manipulation of the immune system, decisively in the host's favor. Neuraminidase immunotherapy in the mouse model system has been shown to accomplish this end. It is hoped that critical insight into basic mechanisms can be garnered from an intensive study of the phenomenon in the murine sarcoma model.

NCP Objectives and Approach: 06, A1, 4.

Proposed Course:

Studies previously mentioned will be pursued. Clinical protocols for human immunotherapy based on findings in the animal model system will be developed.

Publications:

None.

Serial No. Z01-CBD-3814-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of Cryosurgery in Tumor Treatment

Previous Serial Number: Same

Principal Investigators: R. M. Beazley, M.D. and D. H. Bagley, M.D.

Other Investigators: R. E. Terrill

Cooperating Units: None

Man Years:

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Part I. A&B Evaluation of the Effects of Cryosurgery on the Normal Structures of the Upper Urinary Tract

Keyword Descriptors: Cryosurgery, ureters, kidney

Objectives:

Cryosurgery is often used in the treatment of benign and malignant neoplasms. Its early and extensive use in treating tumors of the prostate and bladder has not been extended to the upper urinary tract. This study is designed to evaluate the effect of cryosurgery on the ureters and kidneys.

Part Ia.

Methods Employed:

The left ureter in 14 dogs was surgically exposed to permit cryosurgery of an isolated segment. The cryosurgical dose was varied with alterations in repetitions and durations of exposure. Ureteral function was followed at weekly intervals by excretory urography. Nephroureteectomy was performed on individual dogs at biweekly intervals for histological examination.

Major Findings:

All ureters repetitively exposed to the cryosurgical lesions developed partial obstruction within 2 weeks. Among the 10 dogs followed for more than 4 weeks, stenosis developed in 5 and obstruction in 5. In the dogs receiving single cryosurgical treatments there were no obstructions after the second week.

Progression of fibrosis in the ureter and periureteral tissues was noted. There was no leakage of urine or loss of ureteral integrity. These results show that severe cryosurgical injury to the canine ureter produces scarring with stenosis and obstruction but without loss of continuity.

Part Ib.

Methods Employed:

Renal vascular changes were studied after renal cryosurgery in 15 dogs. In each animal the left kidney was surgically exposed and selective renal arteriogram was performed. The lower pole of the kidney was then frozen for varying intervals with a liquid nitrogen spray. In six dogs intrarenal temperatures were measured with a miniature multiple thermocouple probe. Anteriograms were repeated immediately after complete thaw, and one and six weeks after treatment. In 6 dogs nephrectomy was performed at various periods for histological study.

Major Findings

Cryosurgical treatment of the kidney produced an avascular lesion with subsequent necrosis. The size of the lesion was related to the minimum temperature and the cryosurgical dose. Transient cessation of the arterial flow occurred at -30°C. but irreversal changes were not observed until -50°C. Histologically, the lesion was sharply demarcated from normal tissue.

Significance to Cancer Research and Program of the Institute:

These studies show that cryosurgical treatment of the kidney can produce necrosis. The lesion can be sharply defined and predictably limited. There exists a clear potential for treatment of intrarenal neoplasms, particularly where there is a solitary kidney. However, the results of the study on the ureter show the potential danger of such treatment if it extends to the ureter. Cryosurgical exposure of the ureter can be expected to produce adverse effects with severity depending upon the dose.

NCP Objectives and Approach; 06, A1, A4

Proposed Course:

Renal cryosurgery should be utilized in an animal model with intrarenal tumors. The model should include animals with tumors encroaching upon the renal pelvis. The effects on the ureter have now been defined and care should be taken in treatment of renal lesions to avoid ureteral exposure to cryosurgery.

Publications:

Bagley, Demetrius H., Faraci, Robert P., Marrone, Joanne C., and Beazley, Robert M.: Lymphocyte mediated cytotoxicity after cryosurgery of a murine sarcoma. Jour. of Surg. Res. 17:404-406, 1974.

Faraci, Robert P., Bagley, Demetrius H., Marrone, Joanne C., and Beazley, Robert M.: In vitro demonstration of cryosurgical augmentation of tumor immunity. Surgery 77:433-438, 1975.

In Press:

Faraci, Robert P., Bagley, D.H., Marrone, J.C., and Beazley, R.M.: The effect of curative cryosurgery on the tumor specific immune response of C57 mice. Cryobiology.

Faraci, Robert P., Bagley, D.H., Marrone, J.C., Ketcham, Alfred S., and Beazley, Robert M.: Mechanism of cryosurgicall-induced heightened tumor immunity. The Am. Surgeon.

Ketcham, Alfred S., Beazley, Robert M., and Bagley, Demetrius H.: Cryosurgical treatment of the nervus facialis in the Rhesus monkey. Minerva Oncologia.

Serial No. Z01-CBD-3817-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cryopreservation of Immunocompetent Cells, Tumor Cells, and Cells with Endocrine Activity

Previous Serial Number: None

Principal Investigators: H.F. Sears, M.D.

Other Investigators: S.A. Rosenberg, M.D. and H. Anding

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description: Cryopreservation of Lymphocytes

Keyword Descriptors: Cryopreservation, lymphocyte, macrophage, immunocompetent, tumor cell

Objectives:

The objectives of this study are to determine optimal techniques of cell separation and cryopreservation. Techniques will be developed for the cryopreservation of lymphocytes of tumor cells, and endocrine tissue.

Methods Employed:

Human lymphocytes and other immune competent cells are recovered from samples of normal volunteers' blood, separated by varying techniques including ficoll-hypaque separations, dextran sedimentations, and possibly glass bead or wool column separation. These cells are then washed, counted and introduced to different cryoprotective media including DMSO, human and fetal calf serum, as well as glycerol and sucrose. A controlled rate freezing apparatus employs different freezing rates and a technique of holding cells at predetermined temperatures of below the crystallization points. Differing rates of thawing and methods of handling cells as they are recovered from the frozen state are evaluated. Finally these cells are assessed as to their immediate viability and viability after 24 hours employing trypan blue exclusion and most importantly they are assessed with regard to their ability to retain function for in vitro assays as compared to the initial population prior to freezing. Functional and morphological assays are performed on cells that have been frozen varying lengths of time including those recovered immediately after one day, one week, one month, six months and one year. A similar course of study will be conducted with regard to endocrine gland tissue and tumor tissue.

Major Findings:

This work has just begun and there are no findings as of this date.

Significance to Cancer Research and Program of the Institute:

The preservation of the immune competent cells will allow material to be removed from a cancer patient at a time when the host and tumor are existing together in an unaltered state. Then after different modalities of therapy and more importantly time for either recurrence or residual disease to affect the host-tumor relationship, materials can be again obtained for biologic assays. At this time the biologic assays can be run simultaneously with material harvested at the initiation of the patient's treatment thereby removing many of the inherent variables of most biologic assays.

Endocrine tissue will be removed and preserved in such a way that surgical procedures designed to correct hyperfunctioning states could be more flexible with the quantity of tissue removed when this could be preserved and reintroduced if the patient's state became such that hypofunction of the remaining glands becomes a clinical problem.

NCP Objectives and Approach: 05B, A3, A4, 06, A1, A4

Proposed Course:

Further investigations should be carried out to develop the best techniques for cryopreservation of cells to insure not only viability but function and provide optimal normal response characteristics.

Publications:

None

Serial No. Z01-CBD-3818-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular Immunity in the Tumor Bearing Host

Previous Serial Number: None

Principal Investigator: Max H. Cohen, M.D., Ph.D.

Other Investigators: Edward L. Felix, M.D., J. Milburn Jessup, M.D.
Ronald B. Herberman, M.D., Robert K. Oldham, M.D.

Cooperating Units: Laboratory of Immunodiagnosis, NCI

Man Years:

Total:	1-2/3
Professional:	1-1/3
Other:	1/3

Keyword Descriptors: Immunocompetence, DNCB, skin tests, MIF

Project Description:

Objectives:

The purpose of these studies is to determine the effect of an ongoing delayed hypersensitivity reaction on nonspecific immune reactivity, as measured by lymphocyte reactivity to mitogens. Secondarily, an attempt was made to reverse the immune depression associated with chemotherapy and surgery, since these immune depressions may be deleterious to the cancer patient.

Methods Employed:

Twenty-six patients received topical DNCB, simultaneous with serial measurements of lymphocyte reactivity to phytohemagglutinin. Currently, patients receiving methyl-CCNU chemotherapy are also receiving, on a random basis (according to hospital number), repeated topical applications of DNCB. Other patients, with metastatic cutaneous melanoma, are receiving intralesional injections with BCG or DNCB. Several patients with breast cancer are being followed in conjunction with the Medicine Branch as they receive chemotherapy with or without Corynebacterium parvum. Besides measurements of PHA stimulation of lymphocytes, cytotoxicity assays are being performed and, in conjunction with Dr. Herberman's laboratory, measurements of reactivity to skin test antigens and MIF are being studied.

Major Findings:

Patients undergoing topical application of dinitrochlorobenzene, experience a doubling of the lymphocyte reactivity to phytohemagglutinin. Simultaneous measurement of cell-mediated cytotoxicity in these patients, normal individuals, breast cancer patients, patients with head and neck malignancies, and other tumors, reveals a correlation between the PHA response and the results in the cytotoxicity assays. This may have some relevance to the question of why normal individuals, as a group, tend to have high reactivity against tumor cell lines in some cytotoxicity assays. It is possible that these cytotoxicity assays, at least in part, are simply measuring general lymphocyte reactivity.

The studies on the effect of DNCB on patients undergoing chemotherapy or surgery are ongoing at the present time. The MIF assay, done in conjunction with Dr. Herberman's group, has revealed that an increased percentage of melanoma patients as compared to other patients have reactivity against 3M KCl extracts of melanoma. While these assays seem to be reproducible, the significance of variation over several months of observation, remains to be determined, as does the value of this assay in serial studies on individual patients.

The skin test results at the present time reveal that the crude 3M KCl extracts of breast and melanoma tumors show a significant degree of cross reactivity when tests are carried out on patients bearing tumors of a different histological type than the test antigen. At the present time, studies are being conducted on more purified fractions, which have been passed through Sephadex columns and polyacrylamide gels in order to obtain subfractions, which appear on preliminary testing to have increased specificity, in the few melanoma patients in which it has been tested.

Significance to Cancer Research and Program of the Institute:

It is possible that the immune depression associated with chemotherapy or surgery in cancer patients may be reduced by simultaneous administration of agents which nonspecifically stimulate lymphocyte reactivity. This remains to be determined, as well as the more difficult question of whether these *in vitro* changes correlate with any significant biological effect. It is hoped that specificity in skin testing and in MIF testing will provide a useful method of serial monitoring in patients undergoing multiple modality or single modality treatment.

NCP Objectives and Approach: 02, A1,5,6; 05, A1,3,5; 06, A1,2,4

Proposed Course:

We plan to work toward the goal of continuing to prove or disprove the specificity of reactivity of *in vitro* and skin testing, and to utilize these assays as the patient's immune reactivity is being altered.

Publications:

Cohen, M.H., Chretien, P., Felix, E., Loyd, B., Ketcham, A.S., Albright, L., and Ommaya, A.: Augmentation of lymphocyte reactivity in guinea pigs, mice, monkeys, and humans sensitized to BCG, DNCB, or nitrogen mustard. Nature, 249:656-658, 1974.

Serial No. Z01-CBD-3821-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interactions Between Tumor Immunity and Cellular Immunity to Dinitrochlorobenzene

Previous Serial Number: None

Principal Investigator: J. Milburn Jessup, M.D.

Other Investigators: Max H. Cohen, M.D., Ph.D., Edward L. Felix, M.D.

Cooperating Units: None

Man Years:

Total:	1-1/3
Professional:	1
Other:	1/3

Keyword Descriptors: Cellular immunity, Dinitrochlorobenzene, delayed hypersensitivity

Project Description:

Objectives:

To investigate the effect that a cell-mediated immune response to a chemically-defined hapten such as dinitrochlorobenzene (DNCB) may have upon growth of an experimental tumor and what effect that tumor growth may have upon cell-mediated immunity to DNCB. We will use this data in an attempt to develop methods for analyzing the effect that a patient's immune response may have upon his tumor's growth and the possible modulating effect that cell-mediated immune responses to other antigens may have upon a patient's tumor immunity.

Methods Employed:

A model of delayed hypersensitivity reaction was established in BALB/cAnN mice: 2.0 or 0.2 mg DNCB in 0.10 cc of dimethylsulfoxide (DMSO) was injected intradermally or applied topically to mice. These mice along with untreated littermates, and littermates treated with 0.10 cc DMSO, were injected 10 days later subcutaneously in a hind footpad with 0.05 mg DNCB dissolved in 0.05 cc DMSO. Footpads were then measured 24 to 48 hours later and the results expressed as a percent footpad swelling which was calculated by subtracting the size of the uninjected footpads from that of the injected footpad and dividing the difference by the noninjected footpad.

Tumors employed are transplantable tumors which are carried in ascitic form in BALB/c AnN mice. Meth A is a 3-methylcholanthrene induced fibrosarcoma and Gloria is a radiation induced lymphoma donated by Dr. T. Aoki of the Virology and Immunology Branch, NCI. In experiments designed to test the effect of tumors upon DNCB delayed hypersensitivity, washed ascites cells were injected subcutaneously in the flank and at various intervals thereafter animals were injected in the opposite flank with either DNCB or DMSO and then tested 10 days later with footpad challenge as described above. In experiments designed to test the effect of hypersensitivity to DNCB upon tumor growth, mice were pretreated with DNCB, 2 mg subcutaneously in one flank and then challenged 10 days later with an injection of Gloria or Meth A subcutaneously in the opposite flank.

Major Findings:

Mice pretreated with 2 or 0.2 mg of DNCB had significantly greater footpad swelling than did mice pretreated with DMSO or left untreated but footpad challenged. This footpad swelling was shown histologically to be composed of a predominantly mononuclear infiltrate, was passively transferrable with immune spleen cells but not with immune serum, and was inhibitable by 2.5 mg of cortisone acetate intraperitoneally given simultaneously as the challenge sensitizing injection. In experiments in animals bearing tumors, it was found that animals bearing 1 x 10^6 Meth A cells subcutaneously for 10 days or more had suppression of DNCB sensitivity. However, the amount of swelling in such tumor bearing animals was the same as the amount of swelling seen in normal mice who are unsensitized to DNCB, indicating that the inflammatory response is intact. Similar results were obtained with Gloria lymphoma. Finally, BALB/c AnN mice bearing Cloudman melanoma tumors for more than 50 days also could not be DNCB sensitized but their littermates who had had spontaneous regression of their tumor could be so sensitized. Attempts to elucidate the mechanism of depressed delayed hypersensitivity to DNCB have so far been equivocal. Passive transfer of serum from tumor bearing animals to normal mice does not yield consistent suppression of de novo DNCB delayed hypersensitivity. Transfer of spleen cells from tumor bearing mice to normal mice also does not seem to inhibit DNCB delayed hypersensitivity.

These studies and those with Gloria lymphoma establish an animal model for investigation of the well known clinical finding that patient's with large tumor burden have decreased cell-mediated immunity to DNCB. These studies show that large tumor burdens inhibit DNCB delayed hypersensitivity and allow us an opportunity to investigate the mechanism *in vivo* and *in vitro* of such immune suppression. The enhancement study may allow us an opportunity to investigate how immune responses to different antigens can interact with one another and perhaps influence one another.

Significance to Cancer Research and Program of the Institute:

It is hoped that these studies will provide insight into the mechanism by which tumor depresses cell-mediated immunity and, therefore, give insight into potential therapeutic approaches related to the immune system of cancer patients.

NCP Objectives and Approach: 02, A1,5,6; 05, A1,3,5

Proposed Course:

We propose to investigate the mechanism *in vivo* and *in vitro* by which tumors inhibit DNCB delayed hypersensitivity. Similarly, we will try to determine if it is the immune response per se which causes tumor enhancement in the DNCB pretreatment model and to see if a suppression of *in vitro* responsivity in PHA and MLC would predict the appearance of enhanced tumor growth.

Publications:

Jessup, J.M., Felix, E.L., and Cohen, M.H.: Murine dinitrochlorobenzene (DNCB) reactivity: Depression by melanoma, lymphoma and tumor-associated serum factors. *Proc. Amer. Assoc. Cancer Res.*, 1975, in press.

Serial No. Z01-CBD-3822-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Study of Vitamin A in the Treatment of Murine Tumors

Previous Serial Number: New

Principal Investigator: Edward L. Felix, M.D.

Other Investigators: Max H. Cohen, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1-1/3
Professional:	1
Other:	1/3

Project Description:

Part I. The Prophylactic Use of Vitamin A

Part II. Intralesional Therapy with Vitamin A

Keyword Descriptors: Vitamin A, intralesional therapy

Project Description:

Part I. The Prophylactic Use of Vitamin A

Objectives:

Immune adjuvants such as BCG and C. parvum have been used prophylactically to inhibit the development of tumors in multiple animal models. Other known adjuvants such as Vitamin A might, therefore, be capable of acting as inhibitors of tumor growth and development alone or in combination with other agents. This study was developed in order to determine the effect of oral and intraperitoneal Vitamin A on the development of a murine melanoma.

Methods Employed:

Syngeneic mice were pretreated with either oral or intraperitoneal Vitamin A for a prescribed course. They were then challenged with a uniform dose of S91 melanoma. Animals were then observed and tumor incidence and growth rates were measured. Studies to determine the exact dosage required to inhibit tumor growth were carried out by varying the number of days of treatment or the number of units per treatment. To determine the mechanism of action of Vitamin A, experiments were carried out in which the mice were immune suppressed using steroids and anti-lymphocyte serum.

Major Findings:

Mice pretreated with oral Vitamin A, approximately 3500 units per day, had a significantly lower incidence of tumor development when compared to controls which received normal drinking water ad lib. Mice pretreated with intraperitoneal Vitamin A, 5000 units for five days, also had a significantly lower tumor incidence than did the intraperitoneal saline controls. Dose studies showed that at least 15,000 units of Vitamin A pretreatment was required. When animals were pretreated with steroids, there seemed to be no effect on the Vitamin A protection of the mice. At high tumor doses anti-lymphocyte serum abrogated the Vitamin A protection. At low tumor doses the anti-lymphocyte serum significantly increased tumor incidence over the controls and this enhancement of tumor growth was inhibited by Vitamin A. These experiments, therefore, seem to show a possible interrelationship between Vitamin A and the immune system.

Significance to Cancer Research and Program of the Institute:

These experiments demonstrate that another known immunological adjuvant, Vitamin A, is capable of prophylactically inhibiting the development and growth of a murine tumor. This protection may be the result of immune enhancement as suggested by the studies with anti-lymphocyte serum. The tumor prophylaxis is affected both by Vitamin A dose and size of tumor challenge suggesting a delicate interrelationship between the host immune system and tumor.

NCP Objectives and Approach: 06, A1,4

Proposed Course:

Ongoing studies will further investigate the role of Vitamin A in the prophylaxis and treatment of various tumor systems. The role of the immune system in Vitamin A treatment will be investigated further.

Project Description:

Part II. Intralesional Therapy with Vitamin A

Objectives:

To treat established tumors with an intralesional agent which will cause tumor regression and cure.

Methods Employed:

Syngeneic mice are inoculated between the scapulae in the subcutaneous tissue with a suspension of tumor cells. After observation animals are then selected with uniform tumors and receive intralesional injections of either Vitamin A or saline. The rate of tumor growth and ultimate course are then recorded over a several week period.

Major Findings:

Mice who receive intralesional injections of saline all have steady progression of tumor size and ultimately die from local tumor invasion. Approximately one-third of the Vitamin A treated mice in two different tumor systems have regression and cure of their established tumors. The Vitamin A mice not cured demonstrate a retarded growth rate but ultimately die from tumor invasion.

Significance to Cancer Research and Program of the Institute:

These studies demonstrate that an immune adjuvant, Vitamin A, is capable of causing tumor regression in a murine system in approximately one-third of the cases. It is possible that this agent may be used as a substitute for other intralesional agents known to have severe complications or can be used in combination with these agents to reduce their dose.

NCP Objectives and Approach: 06, A1,2,4

Proposed Course:

Further experiments will be carried out to determine the systemic effects of these intralesional agents as well as their possible mechanism of action.

Publications:

None

Serial No. Z01-CBD-3823-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Experimental Murine Pulmonary Metastases

Previous Serial Number: None

Principal Investigator: W. F. Sindelar, M.D., Ph.D.

Other Investigators: H. Wexler, and D. Jones

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Part I.	Electron Microscopic Observations on the Formation of Experimental Pulmonary Metastases
Part II.	Distribution of Experimental Circulating Tumor Cells
Part III.	Effects of Platelet Inhibitors on Spontaneous Pulmonary Metastases in Murine Malignancies

Keyword Descriptors: Metastasis, experimental pulmonary metastasis, circulating tumor cell, anticoagulation, Warfarin, platelet, platelet inhibition, fibrosarcoma in mouse, melanoma in mouse, electron microscopy.

Project Description:

Part I. Electron Microscopic Observations on the Formation of Experimental Pulmonary Metastases

Objectives:

The early cellular events in the formation of pulmonary metastases are unknown. Much theoretical speculation exists concerning the mechanisms by which hematogenously-disseminated tumor cells attach to pulmonary capillary walls, penetrate the vasculature, and establish metastatic tumor foci within the pulmonary parenchyma. The initial attachment of circulating tumor cells to the capillary endothelium has been regarded as the crucial step in metastasis formation.

The present study represents an attempt to describe by electron microscopy the cellular behavior during early experimental pulmonary metastasis formation in differing mouse tumor systems. Specific areas of descriptive interest are concentrated upon the methods of initial tumor cell arrest within the pulmonary capillary, the role of platelets and fibrin in tumor cell arrest, methods of vascular penetration by arrested cells, effects of anticoagulants on arrest and vascular penetration, and the characteristics of tumor growth at metastatic sites.

Methods Employed:

Both a methylcholanthrene-induced fibrosarcoma (M19B) and a spontaneous melanoma (B16) were investigated. These tumors have been established as transplantable lines in C57BL/6J mice. Preparations of individual dissociated cell suspensions were prepared by trypsinization. In an initial experiment, 75 mice were intravenously inoculated with 1.0×10^6 viable M19B fibrosarcoma cells and were sequentially sacrificed at closely-spaced intervals ranging from 15 minutes to 14 days following cell injection. Lungs were fixed in gluteraldehyde and embedded in Epon. Tissue blocks were serially sectioned to locate the areas containing embolized tumor cells. These areas were thin-sectioned and prepared for examination with the transmission electron microscope. Observations were made on the characteristics of circulating tumor cell arrest within the pulmonary vessel, the methods of cell penetration through the vascular wall, and the pattern of growth of metastatic cell deposits in the perivascular connective tissue. A similar experiment was performed for the B16 melanoma, injecting 150 mice intravenously with 1.0×10^6 tumor cells and fixing lung sections for electron microscopic observation at various intervals up to 14 days after injection. To investigate the influence of anticoagulation on the events of early metastasis formation, 1.0×10^6 B16 melanoma cells were inoculated into 75 warfarin-treated mice, with serial sacrifices made to collect lung tissue for sequential electron microscopic examination. The treated mice received varying doses of sodium warfarin in their drinking water (average dosage - 0.23 mg%) to maintain their prothrombin times at 2-2.5 times the control level. Mice were completely anticoagulated before cell injection (average of 7 days warfarin treatment prior to inoculation with tumor cells), and were maintained on therapeutic levels of warfarin throughout the duration of the experiment.

Major Findings:

Studies on the M19B fibrosarcoma revealed that circulating tumor cells were present in pulmonary capillaries within 15 minutes of injection. Such cells had extensive surface pseudopodia, with which they attached to endothelial membranes during the first three hours after cell injection. Tumor cell attachment to the capillary walls was immediately followed by massive platelet aggregation, creating a dense platelet thrombus at the site of tumor cell embolization. Such platelet thrombi were free of fibrin. The thrombi disintegrated within 18 hours, but the embolized tumor cells continued to adhere to the endothelium by characteristic elongate pseudopodia. Tumor cells then penetrated the capillary walls between 24 and 74 hours after cell injection. Penetration was accomplished through the junctional complex regions joining adjacent endothelial cells. Tumor cells invaded the perivascular connective tissue, where they underwent mitosis to form cell nests that developed into macroscopic pulmonary metastases in all experimental animals within seven days.

Analysis of the cellular events within the pulmonary vasculature following intravenous injection of B16 melanoma are presently underway. However, preliminary observations indicate certain differences in cellular behavior among the B16 melanoma cells as compared with the M19B fibrosarcoma cells.

Observations performed on tissue samples obtained from animals anticoagulated with warfarin are presently being completed. Early data suggests that intravenously-inoculated B16 melanoma cells initially behave similarly in both control and anticoagulated mice. Cells can circulate freely for approximately six hours and can arrest in pulmonary capillaries by means of pseudopodial-mediated adhesions with endothelial membranes. Following cell arrest a slight degree of platelet aggregation occurs. Fibrin thrombi is formed neither in control nor in warfarin-treated animals. An apparent difference between control and anticoagulated animals appeared to be a relative delay in vascular penetration by arrested tumor cells in the warfarin-treated mice. Migratory tumor cells appeared in the pulmonary parenchyma of anticoagulated animals at 24-48 hours, as contrasted with their appearance at six hours or less in the controls. The delay in entering the lung tissue might have resulted either from a prolonged endothelial penetration phase (unlikely since not observed in the specimens so far studied) or from release of initially-arrested cells from the pulmonary vasculature with subsequent re-embolization of the tumor cells and vascular penetration at a later time.

Significance to Cancer Research and Program of the Institute

Information on the behavior of circulating tumor cells as they become arrested in the pulmonary vasculature can indicate possible mechanisms operating in the metastatic process. Any understanding of such mechanisms may provide methods for the ctonrol or prevention of the arrest and growth of hematogenously-disseminated cells to distant organs. It is likely that the characteristics of the events in early blood-borne metastases differ for differing tumors, but basic mechanisms may apply in the crucial steps of attachment of the free tumor cells to the vascular walls of the target organs.

NCP Objectives and Approach: 04, A1, A2

Proposed Course: This project will be completed June, 1975.

Project Description:

Part II. Distribution of Experimental Circulating Tumor Cells

Objectives:

There have been numerous hypotheses concerning the fate of hematogenously-disseminated malignant cells. Some cells give rise to metastatic foci in distant organs. Other cells may remain in the circulation until they die or are destroyed by host defenses. The numbers of such circulating tumor cells in spontaneously-metastasizing neoplasms are unknown, but are generally agreed to be small. Likewise, the fraction of circulating tumor cells which survive to establish metastases in target organs is unknown quantitatively but is believed to be quite low. This study attempts to study the distribution and numbers of cells in various organs following intravenous inoculation of malignant cells in mice, thus providing means to quantitate fate and survival of experimental circulating tumor cells.

Methods Employed:

The B16 melanoma transplantable tumor in C57BL/6J mice was chosen for study. The tumor was adapted for growth in monolayer tissue culture. Such cells grew without difficulty in RPMI 1640 medium supplemented with 20 percent fetal bovine serum, with a doubling time of approximately 36 hours. Cells in culture were labeled with ^{125}I-5-iodo-2'-deoxyuridine (^{125}IUDR) for 24 hours at a concentration of 1μCi/cc medium. Such a procedure resulted in a stable nuclear label (leakage <0.1%; >99% of incorporation into DNA) of high specific activity (average 300,000 dpm/10^6 cells).

Recipient animals were injected intravenously with 1.0 x 10^6 labeled B16 melanoma cells. Animals were sequentially sacrificed at closely-spaced intervals ranging from one minute to seven days following cell injection. Animals were dissected and the radioactivity of various organs was measured. Control experiments established the rate of label reutilization by gut epithelium and bone marrow of the host animals, both rates <1%. The numbers of tumor cells were computed at each time after sacrifice in the following organs: lungs, circulating blood, heart, liver, spleen, kidneys, gastrointestinal tract, and residual carcass. All urine was collected and radioactivity counted, in order to determine the rate of tumor cell death and the excretion of label.

Both normal and warfarin-anticoagulated (prothrombin times 2-2.5 times control) were tested. Sixty animals in each group were injected with labeled cells. Twenty-five control animals were injected with labeled non-malignant fibroblasts. The distributions of embolized cells were compared for all groups.

Major Findings:

Data is presently being analyzed and present conclusions are based on preliminary information.

In normal animals (approximately 80% of the injected labeled B16 cells) are trapped within the lung during the first five minutes following cell inoculation. About 5% of the cells remain in the circulating blood and the balance are scattered throughout the remainder of the organs, in numbers corresponding to the relative blood volumes of the organs. By one hour after injection, significant decreases in the numbers of cells in lung was observed. No increases in the numbers of circulating cells were detected and analysis of ^{125}I excreted in the urine indicated that significant cell destruction (approximately 25% of the injected dose) had taken place. All organs showed progressive decreased in the numbers of embolized cells over the first six hours with the exception of liver. Fraction of cells trapped within the liver increased from about 4% at one minute to 10% by six hours, probably representing the progressive embolization of circulating tumor cells. By 24 hours after cell injection, up to 75% of the inoculated cells had been destroyed, as measured by urinary excretion of ^{125}I. All organs showed progressive loss of label during the first 24 hours, indicating death of embolized tumor cells. At 24 hours, the lungs retained approximately 200,000 cells and the liver about 2,500. All other organs showed no significant numbers of trapped cells. Continued loss of cells was apparent through the first five days, with the lung retaining some 10,000 cells and the liver less than 1,000. Counts then appeared to plateau through seven days, suggesting that the remaining cells in the lung and liver were viable and giving rise to metastatic lesions.

Examination of the results on cell distribution obtained from animals anticoagulated with warfarin suggested no significant difference compared to non-anticoagulated controls, in the initial entrapment of injected cells. During the first hour following labelled cell injection, no significant differences were observed between controls and anticoagulated mice, suggesting that the antimetastatic effect of warfarin was not due to interference with initial tumor cell embolization in the vasculature of target organs. However, beginning at 6-12 hours and continuing through 48 hours after cell injection, a greater rate of cell loss from organs was observed in the anticoagulated animals with the greatest loss of cells from the lungs. This suggested that warfarin was toxic to the embolized tumor cells, causing accelerated cell death or that warfarin interfered with the vascular penetration phase of metastasis formation, causing escape of the tumor cells from vascular channels before they are able to break the vascular walls into the pulmonary parenchyma. By seven days small numbers (3,000-4,000) of residual tumor cells could be detected in the lungs of the anticoagulated animals. This was in fair agreement with control experiments, which showed approximately a 5-fold increase in the number of metastatic nodules in the lungs of normal mice, compared with anticoagulated animals, 21 days following injection of similar numbers of melanoma cells.

Control animals were injected with labeled non-malignant syngeneic fibroblasts. Such cells embolized to the lungs within minutes after injection. However, in contrast to the animals injected with melanoma cells, the fibroblasts were rapidly cleared from the lungs, usually before six hours. No significant numbers of fibroblasts remained in any experimental animals after 24 hours.

Significance to Cancer Research and Program of the Institute:

The study indicates that circulating tumor cells can rapidly embolize to target organs, and that such initial embolization is not significantly affected by anticoagulation. The study further indicates that the vast majority of embolized tumor cells are destroyed. Warfarin anticoagulation does not appear to act by interfering with the initial arrest of tumor cells within the vascular bed of target organs, but rather appears to influence subsequent tumor cell survival or ability to migrate into the parenchyma of host organs. Such information on the behavior of embolized tumor cells can be important in devising treatment modalities aimed at preventing or reducing tumor metastases. From the experimental data, it appears that the most fruitful approach for such modalities might be to aim at increasing tumor cell destruction after embolization, a period where they may be vulnerable to toxic or other agents, rather than preventing the initial arrest of the cells in the capillary bed.

NCP Objectives and Approach: 04, A2

Proposed Course:

This project will be completed June 1975.

Project Description:

Part III. Effects of Platelet Inhibitors on Spontaneous Pulmonary Metastases in Murine Malignancies

Objectives:

Electron microscopic studies of the events in experimental murine pulmonary metastases have indicated that circulating tumor cells initially attach to the target organ capillary endothelium by means of surface pseudopodia. Shortly after attachment the cells aggregate platelets, which form a dense thrombus around the embolized tumor cells, perhaps functioning in mechanically stabilizing the tumor cell's adhesion to the endothelium until it is able to penetrate the vascular wall and invade the organ parenchyma.

The ability to aggregate platelets appeared to vary for differing tumors, being especially great for a methylcholanthrene-induced fibrosarcoma (M19B) and relatively low for a melanoma (B16). An experiment was designed to test the role of platelet aggregation in the formation of spontaneous murine pulmonary metastases by the use of inhibitors which prevented formation of intravascular platelet thrombi.

Methods Employed:

All experiments were performed on adult C57Bl/6J mice. In vitro tests were performed on dissociated cell suspensions of M19B fibrosarcoma and B16 melanoma to determine the tumors' relative ability to aggregate isolated platelets in an aggregometer. M19B fibrosarcoma cells caused massive platelet clumping in vitro, while B16 melanoma cells resulted only in modest platelet aggregation. Cyproheptidine was chosen as a platelet antagonist, following the experimental demonstration that mice treated with daily intraperitoneal injections of cyproheptidine (10 mg/kg) exhibited markedly diminished platelet counts with no significant in vitro aggregation of their platelets. Mice were divided into two groups of 100 animals each. The first group was inoculated with M19B fibrosarcoma intramuscularly, and the second was injected with B16 melanoma cells. The two groups were subdivided into a cyproheptidine-treated (10 mg/kg daily intraperitoneally) and a control (saline daily intraperitoneally) subgroup. Tumors in all animals were allowed to grow until the primaries reached 10 mm in size, 21 days for the fibrosarcoma and 28 days for the melanoma. Animals were then sacrificed. Platelet counts were performed on all animals. Lungs were fixed and the numbers of spontaneous pulmonary metastases counted. Control and cyproheptidine-treated animals were compared for the incidence of lung metastases, both for the fibrosarcoma and melanoma groups.

Major Findings:

For all animals treated with cyproheptidine, mean platelet counts were significantly depressed: control counts 733,000, cyproheptidine-treated 153,000 ($p < 0.001$). Control animals in both the fibrosarcoma and melanoma groups developed frequent metastases. In the cyproheptidine-treated animals, no difference in the frequency of pulmonary metastases compared to untreated controls was observed in the animals infected with B16 melanoma (controls 16/44 with metastases, cyproheptidine 23/45 with metastases; $p > 0.05$). However, for the M19B fibrosarcoma, the cyproheptidine-treated group showed a significantly lower incidence of metastases than the untreated controls (controls 36/49 with metastases, cyproheptidine 15/49 with metastases; $p < 0.001$). Thus, a significant decrease in pulmonary metastases was observed in the presence of platelet inhibition in animals infected with fibrosarcoma, a tumor demonstrated to aggregate platelets both in vitro and within pulmonary vessels at sites of tumor cell embolization. In contrast, no inhibition of metastasis formation was seen in melanoma bearing animals treated with cyproheptidine. Only limited platelet aggregation by melanoma cells was observed in vitro and in pulmonary vessels. It was concluded that intact platelet function is necessary for the establishment of pulmonary metastases for tumors that cause in vitro platelet aggregation. For tumors that do not aggregate platelets, the importance of intact platelet function to the metastatic process appears to be less significant.

Significance to Cancer Research and Program of the Institute:

The demonstration of the importance of platelet function in the metastatic process may have therapeutic significance. If a particular tumor is known to aggregate platelets in vitro, adjunctive treatment of patients bearing such tumors with platelet antagonists may serve to decrease the risk of hematogenously-disseminated metastases during tumor surgery or in the post-operative periods. Obviously such therapeutic significance could only follow the consistent experimental demonstration in a wide variety of tumors of the correlation between tumor-induced platelet aggregation and the diminished incidence of metastases of the tumors in the presence of imparied platelet function.

NCP Objectives and Approach: 04, A2, A3

Proposed Course:

This project will be completed June 1975.

Publications:

Faraci, Robert P.: In vitro demonstration of altered antigenicity of metastases from a primary methylcholanthrene-induced sarcoma. Surgery 76: 469-473, 1974.

In Press:

Wexler, Hilda, Chretien, Paul B., Ketcham, Alfred S., and Sindelar, William F.: Induction of pulmonary metastases in both immune and nonimmune mice: Effect of the removal of a primary tumor. Cancer.

Serial No. Z01-CBD-3824-01-SURG
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Murine and Human Transplantable Tumors in Athymic Mutant Mice

Previous Serial Number: None

Principal Investigators: W. F. Sindelar, M.D., Ph.D., A.S. Rabson, M.D. and G. T. O'Conor, M.D.

Other Investigators: J. Costa, M.D., S. W. Rosen, M.D., and J.M. Lieblich, M.D.

Cooperating Units: Laboratory of Pathology, NCI; Clinical Endocrinology Branch, NIAMDD

Man Years:

Total:	0.50
Professional:	0.25
Other:	0.25

Project Description:

Part I. Studies of Murine and Human Transplantable Tumors in Athymic Mutant Mice

Keyword Descriptors: Nude mouse, human heterotransplantable neoplasms, electron microscopy, Papovavirus, murine C-particle, human chorionic gonadotrophin

Objectives:

The "nude" mouse gene was initially described in 1966, and homozygotes were subsequently found to exhibit thymic hypoplasia with complete deficiency in T-lymphocyte mediated immunologic responsiveness. Nude mice have been shown to accept heterotransplants of both normal and neoplastic tissue. The system shows promise for adaptation to studies of growth and development of various human tumor lines which may be transplanted into nude mice.

Various studies were undertaken to investigate the growth potential in the athymic mice of certain human neoplastic cell lines as well as selected murine tumors. Careful characterizations of the growth patterns might be a significant value if such tumor lines, in particular the human neoplasms, are adapted to studies in nude mice of the tumors' responsiveness to chemotherapeutic or immunotherapeutic manipulations.

Methods Employed:

Female nude mice ranging in age from 6-10 weeks were used for all experiments. The mice were derived from an outbred Swiss stock of mice heterozygous for the nude gene. The athymic mice are exceedingly susceptible to infections, particularly necrotizing hepatitis and fungal infestations of the skin. The mice must be kept isolated. Mortality rate generally averages 10% per week.

Initial investigations were performed on a lymphosarcoma which spontaneously arose in a nude mouse. The tumor was serially transplanted and grown in tissue culture. The tumor was characterized by routine histology, transmission, electron microscopy, karyotype analysis, and molecular hybridization studies.

Two human lymphocytoid cell lines have been successfully inoculated and grown as solid tumors in the athymic mice for several generations. The cell lines were established by culture of surgical specimens of patients with reticulum cell sarcoma (McCoy line) and Hodgkin's disease (Hodgkin's line) Such transplantable tumors have been investigated by electron microscopy and karyotype analysis.

Two functional human carcinomas have been adapted for growth in nude mice and have been carried for over 4 generations by serial passage. Both of the tumor lines were derived from culture of a tumor-containing lymph node of a patient with an advanced lung carcinoma. The tumor produced large quantities of human chorionic gonadotrophin (HCG). An initial tissue culture line was established (Chago line) which continued to produce HCG in culture. A clone was subsequently established from the Chago line (Kim Clone), and maintained in culture. The Kim line produced large amounts of the alpha-subunit of HCG. Both Chago and Kim lines were grown in nude mice. The tumors were studied by electron microscopy, and the serum of tumor-bearing animals was analyzed for HCG and its alpha-subunit.

Primary tissue cultures were established of adult nude mouse kidney cells. Such cultures were infected with a strain of papovavirus isolated from a patient with Wiscott-Aldrich Syndrome (MMV-virus). The cultures underwent malignant transformation and were reinoculated into nude mice. Solid tumors developed and were maintained by serial transplantation. The tumors were studied by electron microscopy, and the serum of tumor-bearing animals was examined for viral-specific T-antibody.

Major Findings:

Study of the spontaneous nude mouse lymphosarcoma revealed typical histological findings of a lymphocytoid malignancy, both at the level of the light and electron microscope. Cells had large regular nuclei and scant cytoplasmic differentiation. After the cell line had been in culture for 6 months, electron microscopy revealed the appearance of typical murine-type viral C-particles, seen in approximately 20% of the cells. Inoculation of the cell cultures back into nude mice produced solid tumors with the histologic appearance of an undifferentiated lymphosarcoma. No viral particles were observed in the solid tumors by electron microscopy. Routine karyotyping showed a murine chromosome complement with a marker chromosome. Molecular hybridization studies showed 40% complementary sequences between RNA extracted from the transplantable solid tumor and DNA complementary to the Rauscher murine leukemia virus.

Two lymphocytoid human cell lines were injected into nude mice, and solid transplantable tumors were established. The solid Hodgkin's line appeared on both light and electron microscopy to have the cytological characteristics of a poorly differentiated lymphoid tumor. Karyotype analysis is in progress. The solid McCoy line had the histological characteristics of a poorly differentiated reticulum cell sarcoma. Electron microscopic observations of the second transplant generation of the McCoy tumor revealed some cells with intranuclear viral-like particles, morphologically resembling the simian oncornavirus group. McCoy karyotype was human aneuploid. No murine C-particles were seen in the Hodgkin's and McCoy lines.

Two solid transplantable tumor lines were established in nude mice from cultures derived from a gonadotrophin-secreting human lung carcinoma. Pathological examination of both the Chago and Kim solid tumor lines revealed histological architecture virtually identical to that of the original tumor, with cords of pleomorphic cells and frequent multinucleated giant cells. No viral particles were seen on electron microscopy. Host animals bearing tumors exhibited gross evidence of gonadotrophin effects including mammary enlargement and hypertrophied uteri. Analysis of serum obtained from tumor-bearing animals revealed high levels of the alpha-subunit of HCG (average of 15 nanograms/cc, with values ranging up to 40 nanograms/cc); non-tumor-bearing control animals had no detectable HCG in their serum.

Primary tissue cultures of nude mouse kidney cells were established. The cells underwent malignant transformation after infection with MMV-virus isolated from a patient with immunodeficiency disease and multiple malignancies. The transformed cells acquired viral-specific T-antigens on their surface membranes (T-antigens homologous to SV-40). Inoculation of the transformed cells into nude mice produced a slow growing but transplantable solid tumor line. Histologically, the solid tumor was composed of myxoid cells in an abundant fibrillar stroma. No viral particles were observed by electron microscopy in either the transformed cell cultures or in the solid tumors.

Significance to Cancer Research and Program of the Institute

The demonstration of C-particles in a tissue culture line derived from a spontaneous nude mouse lymphosarcoma indicates that the athymic mutant animals may harbor an endogenous murine virus. This virus could conceivably contaminate human tumors heterotransplanted into the animals and cause confusion in virological studies on human oncogenesis. The findings indicate that any viruses derived from nude mouse heterotransplants must be carefully distinguished from the endogenous C-particle.

The adaptation of two human lymphocytoid cell lines for growth in nude mice represents the first known successful heterotransplantation of tumors from the lymphoma group into nude mice. Such tumors have potential for use in the study of responses of lymphoid malignancies to various experimental treatment modalities. The findings of viral-like particles in certain cells of the McCoy line are of interest, but the findings are too preliminary to speculate on their significance.

Demonstration of the growth of gonadotrophin-secreting tumors in athymic mice indicates that heterotransplants of human neoplastic tissue retain both their morphological and functional features through serial transplant generations. This finding suggests that the nude mouse represents a satisfactory host for human heterotransplants, causing little artifactual changes in the growth features of the tumors.

The growth of MMV-viral-transformed tumor in the nude mouse represents the first known instance of in vivo growth of oncornavirus-transformed mouse tissue. Previous attempts at reinoculating transformed murine cells have resulted in rapid rejection, due to host reaction against T-antigens on the transformed cells. The nude mouse's immune tolerance apparently allows growth of the viral-transformed cells and indicates that the athymic mutant may be an excellent model for the study of viral oncogenesis in mice.

NCP Objectives and Approach: 01, A4; 03, A2; 04, A1, A3

Proposed Course:

Any available spontaneous tumor arising in nude mice should be subjected to virological studies to determine the frequency of the presence of murine C-particles. It is interesting to note that despite the athymic mutant's T-immunodeficiency, there appears to be no increase in the frequency of spontaneously-arising cancers. Care must be taken when performing experimental work on human neoplasms transplanted into nude mice that no contamination with murine viruses takes place. The nude mouse's immune tolerance to the transplantation of viral-transformed cell lines provides a unique system for studying the growth characteristics of oncornavirus-induced-tumors in mice.

Publications:

None

Serial No. Z01-CBD-3825-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural Demonstration of In Vivo Toxic Effects of Halogenated Anesthetics on Human Hepatocytes

Previous Serial Number: None

Principal Investigator: W. F. Sindelar, M.D., Ph.D.

Other Investigators: None

Cooperating Units: Laboratory of Pathology, NCI; Anesthesiology Department, Clinical Center

Man Years:

Total:	0.25
Professional:	0.25
Other:	0

Project Description:

Keyword Descriptors: Hepatotoxicity, anesthesia, halothane, fluroxene, electron microscopy

Objectives:

Considerable controversy exists over possible hepatotoxic effects of anesthetic agents, and such controversy is naturally of concern to surgeon and anesthesiologist alike. In cancer patients, who are often submitted to numerous operative procedures with anesthesia exposures during the course of their surgical treatment, any risk of anesthesia-induced hepatotoxicity becomes an important consideration, particularly in patients with liver functions which have been compromised by radiation or chemotherapy. A prospective study was undertaken to investigate possible ultrastructural alterations in human hepatocytes, suggestive of cellular toxicity, during exposure to halogenated inhalational anesthetics.

Methods Employed:

The study consisted of 26 patients undergoing elective staging laparotomy for lymphoma, melanoma or ovarian carcinoma. All patients had normal liver function tests preoperatively and had no history nor evidence of hepatic disease.

At laparotomy, all patients were anesthetized with thiopental and maintained on N_2O-O_2-narcotic anesthesia until the surgical incision was completed and a first liver biopsy ("pre-anesthesia") was obtained. After the initial biopsy, the patient was changed to maintenance anesthesia with halothane or fluroxene. After a minimum of 60 minutes exposure to the halogenated anesthetic, a second liver biopsy was taken ("post-anesthesia"). Control patients were continued on maintenance N_2O-O_2-narcotic for both pre- and post-anesthesia biopsies. The pre- and post-anesthesia liver biopsies were examined by electron microscopy.

Major Findings:

Of the 26 patients undergoing biopsies, 21 were suitable for analysis. Five patients were excluded because of gross hepatic pathology. Analysis of 13 patients has been completed; eight are presently being studied.

All of the three control patients studied showed no ultrastructural alterations in hepatocytes between pre- and post-anesthesia biopsies. By contrast all 10 patients analyzed that were given halogenated anesthetics showed evidence of toxic cellular injury developing in the post-anesthesia biopsies. No evidence of hepatotoxic changes were present in any of the pre-anesthesia biopsies. All three patients given fluroxene showed increases in intracellular lipid and blunting of canalicular microvilli in post-anesthesia biopsies. A single fluroxene patient demonstrated partial resorption of mitochondrial crystals. All seven patients given halothane exhibited uniform dilatation of smooth endoplasmic reticulum and blunting of microvilli. Five halothane patients showed glycogen aggregation, and four demonstrated lipid accumulation. No alterations in nuclear morphology, membrane structure, and mitochondrial appearance were observed in any of the post-anesthesia biopsies.

It was concluded that exposure to halogenated anesthetics caused ultrastructural changes suggestive of hepatotoxicity that became apparent after only 60 minutes of exposure. Non-halogenated anesthetics did not cause such changes.

Significance to Cancer Research and Program of the Institute:

The demonstration of alterations in hepatocyte subcellular architecture suggestive of toxic change following exposure to halogenated inhalational anesthetics suggests that such anesthetic agents have a potential for significant liver damage. In the surgical cancer patient, consideration must be given to the nature of the operative procedure to be performed, both in terms of the relative benefits and risks. In the case of a patient with compromised liver function, some thought must be given to possible aggrevation of the liver functions, and possible subsequent hepatic complications, by intraoperative exposure to potentially toxic anesthetics.

NCP Objectives and Approach: 06, A1

This project is being terminated in June, 1975.

Publications:

None

Serial No. Z01-CBD-3826-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Prostatic Tissue Transformed by a DNA Oncogenic Virus

Previous Serial Number: None

Principal Investigator: N. Javadpour, M.D.

Other Investigators: D. N. Danforth, M.D.

Cooperating Units: None

Man Years:

Total: 2-1/2
Professional: 1-1/2
Other: 1

Project Description:

Fetal Antigens and Transformed Prostatic Tissue by a DNA Oncogenic Virus

Keyword Descriptors: Urology, carcinogenesis, prostatic cancer

Objectives:

The relationship between embryonic development and neoplasia has been a central focus of cancer research. The appearance of certain antigens which may be absent from homologous normal tissue have been detected in high concentrations in colonic, hepatic, lung, and testicular cancers. Our purpose is to study fetal antigens in prostatic cancer.

Methods Employed:

We have studied the cross reactivity between prostatic tissue transformed by a DNA oncogenic virus and fetal antigens in hamsters. The LVG-LAK strain of hamster was utilized in experiments. Prostatic tissue obtained from hamsters were grown in tissue culture medium and infected with SV-40 virus. The ability of immunization with fetal antigens to protect against challenge with transformed prostatic tissue was tested.

Major Findings:

Hamsters immunized with fetal antigens demonstrated significant resistance to tumor growth. *In vitro* tests confirmed the presence of cross reactivity between fetal antigens and antigens as tumor tissue.

Significance to Cancer Research and Program of the Institute:

The demonstration of immunologic similarity between fetal antigens and tumor antigens may have important implications for the design of rationale approach to the immunologic diagnosis and treatment of cancer.

NCP Objectives and Approach: 01, A4

These studies will be extended to attempt to isolate and characterize the oncofetal antigens.

Publications:

None

Serial No. Z01-CBD-3827-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Anti-reflux Sigmoid Conduit Versus Refluxing Sigmoid Conduit in Dogs

Previous Serial Number: None

Principal Investigator: N.Javadpour, M.D.

Other Investigators: P. Scardino, M.D.

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1
Other:	1/2

Project Description:

Anti-reflux Sigmoid Conduit Versus Refluxing Sigmoid Conduit in Dogs

Keyword Descriptors: Sigmoid conduit, urinary diversion

Objectives:

To establish the efficacy of anti-reflux operations in preventing pyelonephritis, hydronephrosis and renal damage in dogs.

Methods Employed:

Sigmoid conduits have been constructed in dogs with conventional techniques. The left ureter was anastomosed in a manner that allowed reflux and the right ureter was anastomosed with a submucosal tunnel procedure as an effective anti-reflux procedure. The dogs were studied for evidence of infection and renal damages.

Major Findings:

Non refluxing anastomoses are associated with less infection and renal damage than anastomoses that permit reflux.

Significance to Cancer Research and Program of the Institute:

Data indicates that an anti-reflux sigmoid conduit is superior to a refluxing sigmoid conduit in dogs with urinary diversion. It will be necessary to apply this type of urinary diversion to patients with pelvic malignancies needing urinary diversion.

NCP Objectives and Approach: 07, A2

Proposed Course:

We will continue urinary tract infection to evaluate the long-term effect of anti-refluxing sigmoid conduits in dogs. Preliminary results with the application of such techniques to humans has been encouraging and this program will continue.

Publications:

None

Serial No. Z01-CBD-3828-01-SURG

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An Evaluation of Isolated Pulmonary Allograft Function Using a Canine Model

Principal Investigator: R. N. Scott, M.D.

Other Investigators: A. H. Harken,

Cooperating Units: Cardiopulmonary Surgical Research Laboratory, Walter Reed Army Medical Center, Washington, D.C.

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

Part I. An Evaluation of Isolated Pulmonary Allograft Function Using a Canine Model

Keyword Descriptors: Lung transplantation, pulmonary function studies

Objectives:

To determine those lung function parameters which are of greatest value in alerting the transplant surgeon of impending pulmonary allograft rejection.

Methods Employed:

Currently, there is no ideal technique or device available to permit split pulmonary function testing. Tracheal dividers and modified Carlen's tubes do not provide total right and left lung isolation. Therefore, the first phase of this study will consist of producing a model. The model will be produced by dividing the left main stem bronchus at the carina and bringing it out through a separate left chest incision. Left lung function performed through this left bronchial stoma will thus be totally isolated from the right lung. Following the development of the model, left lung allografts will be performed in which the left main stem bronchus is also brought out a separate left chest incision. Standard technique will be followed for completing the arterial and venous anastomoses. No immunosuppressive agents will be used. Lung function studies will begin immediately postoperatively and will include FRC, TLC, compliance, oxygen consumption, and CO_2 production. The normal right lung will serve as control for pulmonary function.

Major Findings:

Using the technique described above, a satisfactory model has been developed. Several animals have survived for prolonged periods (4-6 weeks) ventilating the left lung via the bronchial stoma. After initial left lung infiltrates, chest roentgenograms cleared progressively. Left lung biopsies at the time of death revealed normal ciliated tracheobronchial epithelium beyond the fifth bronchial division. After a few minor technical imperfections in the model have been corrected, allografts will be studied.

Significance to Cancer Research and the Program of the Institute:

For cancer patients with severe pulmonary insufficiency of any etiology, lung transplantation may be the only opportunity for prolonged life.

NCP Objectives and Approach: 06, A2

Proposed Course:

Further refinement of our model and begin lung function studies in pulmonary allografts.

Publications:

None

Serial No. Z01-CBD-3829-01-SURG

1. Surgery Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Serial Comparison of Silk, Nylon, and Staple-Closed Bronchial Stumps

Previous Serial Number: None

Principal Investigator: R. N. Scott, M.D.

Other Investigators: R. P. Faraci, M.D., A. H. Hough, M.D., and P. B. Chretien, M.D.

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total: 1.5
Professional: 1.5
Other:

Project Description:

Part I. A Serial Comparison of Silk, Nylon, and Staple-Closed Bronchial Stumps

Keyword Descriptors: Inflammation, silk-staple-chromic-nylon suture materials, bronchial stumps.

Objectives:

To evaluate the role of suture-related inflammation in bronchial stump healing following pneumonectomy.

Methods Employed:

Three methods of closing bronchial stumps were evaluated in 95 dogs undergoing left pneumonectomy: interrupted 3-0 silk sutures, interrupted 3-0 nylon sutures, and stapling with an automatic device. The dogs were sacrificed at 24 hours, 3, 5, 7, 10 and 14 days after pneumonectomy. An endotracheal tube was inserted, the left thoracic cavity opened and the right bronchus occluded. The left chest was filled with saline and the endotracheal pressure increased until leakage of air occurred at the bronchial stump. The stumps were removed and examined histologically. The average number of polymorphonuclear leukocytes/high power field at each suture line was determined and collagen levels estimated using a scale of 1+ (immature) to 4+ (mature, solid collagen foci).

Major Findings:

The inflammatory response associated with silk-closed stumps was more severe than staples or nylon at each postoperative interval studied. Differences in the exudative reaction between silk and staple closures were significant on day 5 (238.19 ± 23.28 SD PMN's/HPF in silk closures, 34.95 ± 20.37 SD PMN's/HPF in staple closures) and thereafter ($p<.001$). Inflammatory responses produced by nylon were greater than staples but less than silk. Collagen production appeared first in staple closures (day 3) and also reached peak values earliest (4+ on day 7). Collagen levels in silk closures were consistently less than staple closures and did not reach maximum values until day 10. Collagen levels in nylon closures were intermediate between that of silk and staples. Stumps closed with staples were stronger than silk or nylon on each day studied. The difference in average leakage pressure between staple and silk closures was significant on day 5 (145.3 mm Hg ± 64.9 SD in staple closures, 40.2 mm Hg ± 15.4 SD in silk closure) and thereafter ($p<.001$). Nylon leakage pressures were intermediate between silk and staples.

Significance to Cancer Research and the Program of the Institute:

These studies suggest that inflammatory responses correlate with strength of bronchial stump closure. Surgeons should consider this in their selection of suture materials for bronchial stump closure following pulmonary resections.

NCP Objectives and Approach: 06, A2

Proposed Course:

Project completed.

Publications:

Scott, R.N., Faraci, F.P., Goodman, D.G., Militano, T.C., Geelhoed, G.W., and Chretien, P.B.: The role of inflammation in bronchial stump healing. Ann. Surg., April 1975.

SUMMARY

Tumor Immunology Contract Program
July 1, 1974 through June 30, 1975

An Immunology Program Plan has been designed which has as its goals 1) the use of immunologic techniques to detect agents etiologic for cancer, 2) the prevention of cancer by immunologic manipulations, 3) the use of immunologic techniques to improve the diagnosis of cancer, and 4) the manipulation of the immune system of patients with established cancer so that increased survival is achieved.

The Immunology Contract Program has been designed to achieve these goals through the support of investigations relevant to the Plan. Thus far, implementation has been directed toward 1) studies of fundamental problems (designated immunobiology), the solutions to which are required for achieving the four goals, 2) immunodiagnostics, and 3) immunotherapy. All projects have been assigned to one of these three categories although many projects include research in more than one category.

A. IMMUNOBIOLOGY

1. Identification and characterization of surface membrane components of tumor cells and cells of the immune system. Projects in this area include studies of (1) tumor associated antigens and corresponding antibodies (43921, 43858); (2) biochemical, serologic and immunogenetic characterization of normal and tumor cell surface antigens (43920, 43986, 43925, 43985, 44009, 44005; (3) organization and dynamics of cell surface membrane components relevant to tumor immunology (33879, 43922, 43987, 44008, 44000).

2. Investigations of reactivity of hosts to tumors and identification of cells involved. Projects in this area include studies of (1) differentiation of immune cells and determination of roles of different components in immunologic reactions to tumors (23889, 33854, 33859, 33866, 33868, 33870, 23890, 53868, 43926, 43927, 43970, 43929, 43932, 43971, 43994, 43997, 43998); (2) antigen binding by T-cells and transplantable T-cell tumors (43923, 43999, 43924); (3) mechanisms for tumor cell destruction and means of escape from these (43930, 43931, 44001; (4) tumor neo-vascularization (43942) and (5) genetic control of immune responses in relation to cancer (43934, 43972).

3. Development, maintenance and distribution of special experimental animals' tumors and cell lines and development or improvement of techniques needed in tumor immunology. Projects in this area include (1) maintenance and distribution of mouse tumors and cell lines (23886); (2) development and distribution of H-2 recombinant strains of mice (43941, 43935, 43988, 43936); (3) development of improved techniques for in vitro immunologic studies (23883) and (4) development of a bulk electrophoretic method for separation of sub-populations of lymphocytes (43928).

B. IMMUNODIAGNOSIS

1. Evaluate use of carcinoembryonic and other fetal antigens in the diagnosis of human cancer. Projects in this area include (1) development of more sensitive assays (CB-23890); (2) attempts to better understand the chemical composition and structurally characterize fetal antigens (CB-23877, CB-33869, CB-33264, CP-43277, CB-43989); and (3) clinical studies of usefulness in diagnosis or monitoring of patients with malignancy (CB-33858, CB-3387[illegible], CB-[illegible], CB-43888).

2. Search for new tumor associated antigens in human cancer which may be useful in immunodiagnosis. Projects in this area include (1) animal models for circulating tumor associated antigens (CB-43891); (2) investigation of other extraction procedures for glycoproteins from gastrointestinal cancer (CB-43859); (3) extraction of antigens and immunization of heterologous species to develop new potentially useful diagnostic reagents (CB-43860, CB-43889, CB-43958, CB-43890).

3. Evaluate diagnostic usefulness of serologic assays and of humoral immune response to tumor associated antigens. Projects in this area include (1) develop animal models and more sensitive techniques for early detection of antibodies to tumor associated antigens (CB-43882); (2) identification of antibodies to autologous human cancer (CB-43881, CB-43953); and (3) serum collections for evaluation of serum diagnostic tests (CB-23879, CB-33914, CB-43861, CB-33915).

4. Evaluate diagnostic usefulness of cell-mediated immune response to tumor associated antigens. Projects in this area include (1) improved methodology of assays for cell-mediated immunity (CB-43965, CB-43893); (2) improved procedures for preparation of effector lymphocytes and target cells (CB-43854, CB-43964, CB-43885, CB-43883); (3) isolation of antigens active in *in vivo* and *in vitro* assays of cell-mediated immunity (CB-92176), and (4) evaluate usefulness of assays for depression in cell-mediated immunity in cancer patients (CB-43853, CB-43856).

C. IMMUNOTHERAPY

1. Preclinical Research. Animal models are being used to develop information regarding the therapeutic parameters of dose, route and schedule of administration of agents such as BCG (23221, 33891, 33896), its methanol extractable residue (MER) (02208), C. Parvum (23221), and neuraminidase (VCN) treated tumor cells (33860, 43864, 43865). Subcellular fractions such as transfer factor (TF) and immune RNA (iRNA), as specific immunotherapeutic agents, represent a special example of the use of animal models to optimize therapeutic parameters (43949) by the inclusion of subhuman primates (43951). Early evidence suggests this last system may eventuate in therapeutic and tumor models of much greater relevance to man than those available in the usual inbred mice.

A significant portion of the preclinical program is directed towards evaluation of promising new immunotherapeutic approaches. Early evidence indicates that defined chemical substances may improve immunologic response to tumors (43872, 43887). Similarly, chemicals may be used to increase the antigenicity of tumor surfaces (43967). Hybridization of tumor cells to non-malignant cells has shown early promise as a method of production of a specific immunotherapeutic material (43876).

2. Immunologic Status of Recipients of Immunotherapy. Studies of immunotherapeutic agents *in vivo* usually have, as an integral effort, *in vitro* evaluation of immunologic responses directed towards understanding and correlating therapeutic results with host immune response. Other contracts have a primary focus on this aspect of immunotherapy (43887, 43873, 43948, 43874). Early results suggest a consistent pattern to the effect that activated macrophages can inhibit (though not necessarily kill) tumor cells in a non-specific manner and that a common feature of non-specific immunotherapeutic agents is the activation of host macrophages.

The ability to follow immune parameters in human recipients of immunotherapy will be enhanced by a contract to simplify enumeration of B and T cells in peripheral blood (43875) and simplify reproducible application of standardized recall skin test antigens (43954).

3. Human Studies. As a consequence of preclinical studies and human trials utilizing presently available supplies of BCG, a new method of producing BCG in volume has been developed (23221). This material contains no foreign animal protein and is greatly increased in viability per unit weight. It is now ready for trials in human patients under an Investigational New Drug permit (53885). One contract (33888) has contributed to the early but consistent evidence in support of the benefits of non-specific immunotherapy in acute myelogenous leukemia. This same contract plus one other (43852) have early evidence in controlled trials which supports preliminary data suggesting the usefulness of BCG in melanoma. Feasibility studies regarding aerosol administration of BCG in lung cancer patients have been completed and a controlled trial has been initiated (43857), as is true for transfer factor therapy of sarcoma (43878).

Contracts for the evaluation of neuraminidase treated tumor cells in the therapy of advanced cancer (23885) and the effect of deblocking plasma on the immune system of patients with melanoma (23887) are nearing the stage where their results may be evaluated. Topical application of immunotherapeutic agents (33373) continues to offer new leads of possible systemic approaches to immunotherapy. An ongoing contract is evaluating the combination of MER and neuraminidase treated allogeneic tumor cells in acute myelogenous leukemia (43879).

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. J. Claude Bennett University of Alabama in Birmingham Birmingham, Alabama 35294

Contract Number: N01-CB-43892

Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: Using the TL antigens system of the mouse, the contractor will prepare and purify cell surface antigens and perform relevant biochemical studies. An attempt will be made to develop methodologies with wide applicability in studies of cell surface macromolecules.

Progress: Procedures have been developed for preparing serologically pure TL antigen, and quantitative documentation of specific activity of TL antigen at each stage of column fractionation has been made. Subfragments of papain-solubilized TL have been labelled and separated on gels, and molecular weights have been determined. Microchemistry for amino acid sequencing is being pursued, and micro-chemistry for carbohydrates is being evaluated.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $95,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator: Name/Address Performing Organization:
Frank Lilly, Ph.D.
Albert Einstein College of Medicine
Bronx, New York 10461

Contract Number: N01-CB-43934
Starting Date: 5/16/74
Expiration Date: 5/15/75

Goal: To identify single genes and/or polygenic systems involved in immune responses and to determine their relationship to tumor immunity.

Approach: The contractor will study various strains of mice for responsiveness to H-2 D.2 antigen. Congenic strains will be used to identify H-2 linked and H-2 independent genes involved in the response.

Progress: Progress is being made in determining why B10 A (5R) mice do not make cytotoxic anti-H-2D^b antibodies when immunized with B-10 normal spleen cells.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology
Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Analysis of Serum Requirements for *In Vitro* Immunological Studies

Principal Investigator: Name/Address Performing Organization:	Robert I. Mishell, M.D. University of California, Berkeley Berkeley, California 94720

Contract Number: N01-CB-23883

Starting Date: 6/28/72 Expiration Date: 8/75

Goal: To define serum requirements for immunological studies employing tissue culture techniques.

Approach: Using the mosue spleen *in vitro* immune response as an assay, sera obtained from several species will be examined for their capacity to substitute for fetal bovine sera. The latter are in very limited supply. In addition, attempts will be made to explore the use of bacterial endotoxin and other agents for their capacity to restore batches of sera that are not useful in the *in vitro* assays. Furthermore, preliminary fractionation and characterization of both toxic and growth promoting substances will be carried out.

Progress: Ninety-seven lots of fetal bovine sera were screened for ability to support an *in vitro* primary humoral response. Studies of inhibitory effects of newborn calf serum and normal mouse serum on *in vitro* humoral responses were also performed.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Most *in vitro* studies of cellular immune processes depend on added serum. It is essential that sources of such sera be standardized in order to properly evaluate cancer patients' responses to therapy.

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date: April 25, 1972

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Eli E. Sercarz
University of California
Los Angeles, California 90024

Contract Number: NO1-CB-43972

Starting Date: 6/01/74 Expiration Date: 10/31/75

Goal: To identify single genes and/or polygenic systems involved in immune responses and to determine their relationship to tumor immunity

Approach: The contractor will study the mechanisms by which the immune response to gallinaceous lysozymes is regulated at the genetic level in the mouse.

Progress: New contract.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $80,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Development or Improvement of Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Name/Address Performing Organization: Paul Terasaki, Ph.D.
University of California
Los Angeles, California 90024

Contract Number: N01-CB-43884
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To develop improved methods for in vitro sensitization of human lymphocytes, directed at producing high yields of cytotoxic cells.

Approach: The contractor will evaluate the efficacy of various immunization methods, measuring release of ^{51}Cr from cultured human tumor cells. Variables will include target cell types, treatment of immunizing cells, types of responding cells, adjuvants, and culture conditions.

Progress: Attempts have been made to optimize conditions under which maximal in vitro sensitization can be obtained, and a number of variables have been studied. Efforts have been concentrated on using long term cell culture lines as target cells with variable results.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $53,960 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Donald A. Rowley, M.D.
Name/Address University of Chicago
Performing Chicago, Illinois 60637
Organization:

Contract Number: N01-CB-43998
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will prepare anti-receptor antibody (ARA) and measure the effect of ARA on immune responses.

Progress: Reagents and assays necessary for the work proposed have been developed and the investigators are about to begin experiments with anti-receptor antibody.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Imunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $55,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator: Name/Address Performing Organization:	Dr. Richard A. Rifkind College of Physicians and Surgeons of Columbia University New York, New York 10032

Contract Number: N01-CB-43880

Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will study cell surface properties associated with viral transformation of erythroid precursor cells and the effects of such alterations of surface properties on differentiation and proliferation.

Progress: Studies of cell surface alterations of Friend-virus-infected erythroid precursor cells have been initiated, including investigation of lectin responses, enzyme reactions, and changes in electrostatic charge. An attempt is also being made to characterize genetic variants of Friend-virus infected cells.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $51,094

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator: Name/Address Performing Organization:	Dr. Jack H. Stimpfling Columbus Hospital Great Falls, Montana 59401

Contract Number: N01-CB-43935

Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To facilitate the development of suitable production and distribution centers to provide H-2 recombinant or mutant congenic mice in adequate numbers for immunogenetic studies.

Approach: The contractor will produce and distribute to other qualified investigators up to 100 mice per month of at least 5 different H-2 recombinant strains of mice.

Progress: More than 900 congenic recombinant mice have been provided to 18 investigators to date. A notice of availability of 21 recombinant lines has been sent to Mouse Newsletter and to 100 investigators.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $21,088

CONTRACT RESEARCH SUMMARY

Title: Isolation, Characterization and Immunochemistry of Tumor Angiogenesis Factor (TAF)

Principal Investigator:	Bert L. Valle
Name/Address	Harvard Medical School
Performing Organization:	Boston, Massachusetts

Contract Number: N01-CB-43942

Starting Date: 3/1/74 Expiration Date: 2/28/75

Goal: To purify and characterize TAF and produce the corresponding antibody.

Approach: TAF will be purified to homogeneity and characterized as to chemical composition. Throughout purification in vivo bioassays will be carried out to confirm its specific activity, i.e. ability to induce neo-vascularization.

Progress: Techniques have been developed for extracting TAF (Tumor Angiogenesis Factor) from tissue culture cells and production of this material has been substantially increased. Assay procedures for the material have been refined.

Significance for Cancer Research (NCP Objective 4 Approach 2)

If neo-vascularization could be prevented by antibody against TAF, tumor growth might be prevented.

Project Officer: Barbara Sanford, Ph.D.
Program: Immunobiology Site Visit Date: October 5, 1973
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $175,656 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T-Cells

Principal Investigator: Name/Address Performing Organization:	V. E. Olavi-Makela Helsinki University Helsinki, Finland

Contract Number: N01-CB-43923

Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To investigate the properties of antigen-binding receptors on antigen-activated T lymphocytes.

Approach: The contractor will study the fine specificity of various manifestations of acquired immunity and will also undertake studies on genetically controlled differences in fine specificity of anti-NP antibodies.

Progress: These investigators have identified a heavy chain V-gene controlling the fine specificity of anti ABA-HOP in the mouse and have obtained evidence that ABA-TYR can act as a helper determinant in T cell-B cell collaboration, have studied the fine specificity of ABA-TYR induced antibodies and MIF priming in rats, and have observed that heavily haptenated NNP cells formed rosettes with almost all non-T lymphocytes.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $29,000

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator: Name/Address Performing Organization:	Marianna Cherry, Ph.D. Jackson Laboratory Bar Harbor, Maine 04609

Contract Number: N01-CB-43988

Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To facilitate the development of suitable production and distribution centers to provide H-2 recombinant or mutant congenic mice in adequate numbers for immunogenetic studies.

Approach: The contractor will make available for distribution mice of 8 H-2 recombinant congenic strains, 3 H-2 mutant congenic strains and 3 inbred partners of the congenic strains. Genetic testing will be carried out within lines and between appropriate lines to screen for recent mutations.

Progress: After suitable genetic testing and quarantine procedures, 7 H-2 recombinant congenic strains, 3 H-2 mutant congenic strains and 3 inbred partner strains have been transferred into the Jackson Laboratory production facility. Availability of these mice for distribution to investigators was announced in Mouse Newsletter.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $20,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Mechanisms for Cell Mediated Destruction of Tumor Cells

Principal Investigator:	Dr. Christopher S. Henney
Name/Address	Johns Hopkins University
Performing Organization:	Baltimore, Maryland 21218

Contract Number: N01-CB-43995
Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To study early cell surface changes associated with destruction of tumor target cells.

Approach: The contractor will evaluate the protein synthetic requirements for cytolysis, studying the effects of various inhibitors and the kinetics of the efflux of material of various molecular sizes from damaged target cells.

Progress: Considerable progress has been made on developing a reproducible quantitative assay for the measurement of non-T cell mediated cytolysis.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $49,669 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator: Name/Address Performing Organization:	Dr. Michael Edidin Johns Hopkins University Baltimore, Maryland 21218

Contract Number: N01-CB-43922

Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will fuse mouse and human cells of normal and transformed lines and study antigen mobility.

Progress: Fibroblast cultures have been established from 12 mouse congenic-resistant mouse strains, including 5 H 2 recombinants, and fusion experiments are underway.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $55,469

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Name/Address Performing Organization	Dr. Saul Roseman Johns Hopkins University Baltimore, Maryland 21218

Contract Number: N01-CB-54985
Starting Date: 5/16/74 Expiration Date: 5/15/75

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate cell surface components involved in intracellular adhesion, prepare antibodies against these specific molecules and use such antibodies in studies of effects on the adhesive process per se, time of appearance of antigens during embryonic development and relationship with other cell surface components.

Progress: Among the findings so far has been the observation that glutaraldehyde fixed cells adhere to normal cell layers, while kinetics and other properties of the system resemble in many ways those of normal cell - cell adhesion.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $69,414 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: The Role of T-lymphocytes in Tumor-Associated Immunity

Principal Investigator: Name/Address Performing Organization:	Dr. Birger Andersson Karolinska Institute S-104 01, Stockholm 60, Sweden

Contract Number: N01-CB-33866

Starting Date: 3/25/74 Expiration Date: 3/24/75

Goal: To evaluate the role of B cell immunity in *in vivo* tumor rejection using specially prepared animals.

Approach: Tumor rejection capacity will be evaluated by innoculation of titrated tumor doses and measurement of antibody production. At least three well-defined tumors will be used in these assays.

Evaluation of T cell effects will be made utilizing at least two different methods of T cell reconstitution.

In utilizing bone marrow for B cell reconstitution, effort will be made to evaluate or eliminate the presence of contaminating T cells.

Progress: Thymectomized mice reconstituted with mature B cells, T cells, or B and T cells have been used in studies of the effector phase of the immune response to Moloney Leukemia Virus induced tumors in mice. Cell mediated antibody dependent cytotoxicity was shown to be induced by tumor specific IgM antibody as well as by IgG antibody. Model systems with chemically well-defined antigens such as BSA were also studied.

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{2\ \&\ 3}{1\ \&\ 4}$

Understanding the role of types and relative numbers of T and B cells in immune reactions is basic to manipulation of immune defense against cancer.

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunobiology Coordinating Committee

FY75 Funds: $20,000

CONTRACT RESEARCH SUMMARY

Title: Cell Synergy in Cell Bound Immune Reactions

Principal Investigator:	Dr. Henric Blomgren
Name/Address	Karolinska Institute
Performing	S-104 01, Stockholm 60, Sweden
Organization:	

Contract Number: N01-CB-33868
Starting Date: 3/25/74 Expiration Date: 3/24/75

Goal: 1) To evaluate synergism between thymus cells and lymph node cells in graft versus host and tumor rejection reactions, and 2) to evaluate T cell tolerance after passage through allogeneic hosts.

Approach: Project 1 will evaluate the interrelations between thymocytes and lymph node cells in several situations as follows: a) synergistically in graft versus host responses, b) antagonistically in graft versus host responses, c) synergistic effects on the rejection of allogeneic tumors, and d) synergy in reactions between thymocytes and lymph node cells in the mixed lymphocyte reaction (MLR). In particular, the kinetics of regenerating thymus cells, their circulating characteristics, and their PHA responsiveness will be evaluated.

Project 2 will be a study of the development of immunologic tolerance in T cell populations passaged through allogeneic hosts. A second part of this project will be evaluation of the specific tolerance that can be seen in the donor bone marrow that is used to reconstitute lethally irradiated animals shortly after irradiation. In particular, the duration of tolerance will be evaluated and the question of whether bone marrow from such a chimera can be used to protect lethally irradiated mice syngeneic with the marrow will be examined.

Progress: Investigators on this project are making excellent progress with two related problems, one involving a soluble mitogen released by peripheral lymphocytes when stimulated by a phytomitogen and the other involving dissociation of humoral and cellular responses.

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{3\ \&\ 4}{1\ \&\ 4}$)
Understanding the role of types and relative numbers of T and B cells in immune reactions, including tolerance, is basic to manipulation of immune defense against cancer.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $30,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens and Corresponding Antibodies

Principal Investigator: Name/Address Performing Organization:	Dr. Eva Klein Karolinska Institute Stockholm, Sweden
Contract Number: N01-CB-43921	
Starting Date: 6/1/74	Expiration Date: 5/31/75

Goal: To learn more about antigens and antibodies related to malignant transformation, for potential applicability to development of diagnostic tests and therapeutic approaches for cancer.

Approach: The contractor will attempt to identify and characterize tumor associated antigens and antibodies in sarcoma, carcinoma and glioma patients. The immunoglobulin coating of tumor cells will also be studied.

Progress: Cytotoxic effects mediated by lymphocytes from cancer patients after in vitro "education" on autologous tumor cells have been investigated and appearance of non-specific killer cells has been reported.

Significance for Cancer Research (NCP Objective 3 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Effector Mechanisms in Tumor-Associated Immune Reactions

Principal Investigator: Name/Address Performing Organization:	Dr. Eva Klein Karolinska Institute S-104 01 Stockholm, Sweden

Contact Number: N01-CB-33870

Starting Date: 6/25/73 Expiration Date: 3/74

Goal: To study the mechanisms of B cell action in tumor systems.

Approach: At least 6 different model systems shall be used for the following studies: 1) the role of antibody effect in cytotoxicity, 2) Burkitt lymphoma and mouse lymphoma in nude mice, 3) human B cells in relation to Burkitt cell in culture, 4) human glioma patients' lymphocytes in relation to glioma cell lines, 5) Marek's disease, and 6) evaluation of human chronic lymphatic leukemia cells for capacity to act as cytotoxic cells.

Progress: Studies are being pursued on natural killer activity of syngeneic or semi-syngeneic mouse spleen cells against in vitro maintained Moloney lymphoma lines, and it has been shown that there is a strong genetic component to the reaction. Other studies involve cross-reactivity of methylcholanthrene-induced tumors, immunoglobulin coating of human lymphoma cells in vivo, and effector and target studies on established lymphoblastoid cell lines.

Significance for Cancer Research (NCP Objective 3 Approach 4)

	Objective	Approach
	3	4
	6	1
	6	4

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $45,000

CONTRACT RESEARCH SUMMARY

Title: Roles of Purified Cells and Antibodies in Cell-mediated Lysis

Principal Investigator: Name/Address Performing Organization:	Dr. Hans Wigzell Karolinska Institute S-104 01, Stockholm 60, Sweden

Contract Number: N01-CB-33859

Starting Date: 3/25/74 Expiration Date: 3/24/75

Goal: To evaluate several different proposed methodologies for the purpose of achieving high concentrations of T cells and B cells independently of each other.

Approach: The following methodologies will be thoroughly evaluated: 1) the use of antigen coated bead columns to cause adherence of B cells with the subsequent use of dextranase to dissolve the beads, 2) selective cytolysis of T cells with specific anti-T cell antibody, and 3) the evaluation of monolayers of appropriate target cells to achieve adherence of specifically sensitized lymphocytes.

For monitoring, at least three target systems will be used, chick red blood cells, tumor cells (human or mouse), and histoincompatible fibroblasts or tumor cells.

Progress: Progress on improving techniques for purifying subpopulations of lymphoid cells has been excellent, and a variety of techniques have been used effectively, namely (1) rosette sedimentation, (2) selective cytotoxicity, (3) specific adsorption of cells to monolayers and (4) affinity chromatography on beads.

Significance for Cancer Research (NCP Objective 3, 6 Approach 3 & 4)

The ability to work with purified populations of immunocompetent cells is basic to understanding and manipulating immune control of cancer.

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $58,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen-Binding Activity of Transplantable T-Cell Tumors

Principal Investigator:	Oliver A. Roholt, Ph.D.
Name/Address	Health Research Inc.
Performing	Roswell Park Memorial Institute
Organization:	Buffalo, New York 14203

Contract Number: N01-CB-43924
Starting Date: 6/16/74 Expiration Date: 6/15/75
Goal: To develop animal models of transplantable T-cell tumors possessing known specific antigen binding capacity.

Approach: The contractor will perform a pilot study, screening a minimum of 15 transplantable T-cell tumors for specific antigen-binding sites. The technique used for detection of antigen binding will be the paired iodination procedure.

Progress: Ten tumors have been tested for binding against 6 to 14 antigens, and several showed possibly significant binding relationships.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $13,000

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Name/Address Performing Organization:	Harold F. Dvorak, M.D. Massachusetts General Hospital Boston, Massachusetts 20014

Contract Number: N01-CB-43894

Starting Date: 5/16/74 Expiration Date: 1/31/75

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate, purify and chemically characterize cell coat material of normal and activated macrophages, ascites tumor cells and normal and transformed fibroblasts.

Progress: Contract has been terminated. Normal macrophage cell coat material has been isolated, purified and chemically characterized. Studies have also been initiated on cell surface material of normal and transformed hamster cells.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: Contract is being terminated.

CONTRACT RESEARCH SUMMARY

Title: Identification, Separation, Quantitation and Characterization of Lymphocytes and Macrophages

Principal Investigator: Name/Address: Performing Organization: Dr. Nicholas Catsimpoolas
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Contract Number: N01-CB-43928
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To develop improved methods of quantitatively recovering and identifying, separating, quantitating and characterizing sub-populations of lymphocytes.

Approach: The contractor will develop a bulk electrophoretic method for separation of specific sub-populations of surface antigen receptor bearing lymphocytes.

Progress: Progress has been made toward construction of a computerized analytical electrophoresis instrument, and several essential components have been designed and built. Construction of the optical unit and the electrophoresis cells is in progress.

Significance for Cancer Research: (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit: April, 1974
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $71,441 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Serologic and Immunogenetic Investigations of Tumor Associated and Normal Cell Surface Antigens

Principal Investigator: Name/Address Performing Organization:	Ian McKenzie, M.D., Ph.D. Melbourne University Victoria, Australia

Contract Number: N01-CB-43925
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To develop new test systems and serologic reagents for detection and characterization of cell surface antigens relevant to tumor immunobiology.

Approach: The contractor will study Ly-4.2 in normal and tumor tissues and will determine the correlation of Ly-4.2 with other B cell markers. Studies of other Ly antigens will also be undertaken.

Progress: Preparation of Ly-4.2 antisera has continued, and preparation of anti Ly-4.1, anti Ly-5.2, anti Ly-6.2, and anti Ly-7.1 has been initiated, and the search for new specificities is being continued.

Significance for Cancer Research (NCP Objective _3_ Approach_5_)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Improved Detection and Development of H-2 Recombinant Strains

Principal Investigator: Name/Address Performing Organization:	Dr. Donald C. Schreffler University of Michigan Medical School Ann Arbor, Michigan

Contract Number: N01-CB-43936
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To develop improved methods for studying mutation and recombination within the H-2 locus of the mouse, as a model for studying membrane changes in carcinogen or virus induced cancers.

Approach: The contractor will develop an extended series of H-2 recombinant or mutant congenic strains developed from a few key haplotypes. Screening will be done initially by serologic techniques, skin grafting and MLR, with such techniques compared to establish the most effective methods.

Progress: Comparisons of efficiencies and sensitivities of 3 methods of screening for H-2 recombinants are underway.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $20,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells In Tumor Masses

Principal Investigator: Name/Address Performing Organization:	Dr. John W. Kreider Milton S. Hershey Medical Center Hershey, Pennsylvania

Contract Number: N01-CB-43931
Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To provide information about the numbers of immune-related cells in tumor masses, their identification, function in the tumor mass and factors influencing their movement.

Approach: The contractor will quantitate and identify immunocytes in tumor mononuclear infiltrates, relating the ratio of lymphocytes of all types to tumor cells with *in situ* rates of death and proliferation. Immunocyte functional activity will be assessed *in vivo* and *in vitro*.

Progress: Contractor has made substantial progress toward developing the necessary reagents and assays for this project.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $70,438 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator:	Dr. Yves Borel
Name/Address	New England Medical Center
Performing	171 Harrison Avenue
Organization:	Boston, Massachusetts 02111

Contract Number: N01-CB-43970
Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study the interaction of lymphoid cells, their membrane receptors, and their specific antigenic determinants during the induction and maintenance of immunologic tolerance.

Progress: Tolerogens and immunogens have been found to differ in their binding avidity for the receptors on hapten binding cells and evidence has been obtained that "receptor blockade" is directly related to the ability of a hapten to induce tolerance.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Name/Address Performing Organization:	Ross S. Basch, M.D. New York University Medical Center New York, New York

Contract Number: N01-CB-43927
Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To increase understanding of lymphoid cell differentiation, which is fundamental in investigations of immunologic responses to tumors.

Approach: The contractor will attempt to isolate, identify and determine the state of maturation of thymin-sensitive pre-T cells and to establish the mechanism through which thymin acts to convert these to cells resembling intra-thymic T cells.

Progress: New evidence has been obtained on the role of thymopoetin, and a quantitative assay has been developed for studying bone marrow and splenic precursors of the cells which repopulate the thymus of heavily irradiated mice after transplantation. Studies are also underway on the degree of differentiation induced by thymopoetin treatment of the precursor cell population.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $51,568

CONTRACT RESEARCH SUMMARY

Title: Chemical Characterization of Purified Thymic Products or Other Agents Promoting Lymphocyte Differentiation

Principal Investigator: Name/Address Performing Organization:	Dr. Gideon Goldstein New York University Medical Center New York, New York

Contract Number: N01-CB-53868
Starting Date: 8/15/74 Expiration Date: 8/14/75

Goal: To characterize thymic factors which affect differentiation of lymphocytes.

Approach: Thymopoetin, the factor originally described by this investigator is being chemically characterized.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program Immunobiology Site Visit Date: June, 1974
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $98,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: John H. Schwab, Ph.D.
Name/Address Performing Organization: University of North Carolina
Chapel Hill, North Carolina

Contract Number: N01-CB-43997
Starting Date: Expiration Date:
Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study the effects on the immune response of defined components of group A streptococcal cells and determine whether these can affect tumorigenesis or tumor growth.

Progress: Effects of defined group A streptococcal cell components on the immune system have been studied and it has been found that either stimulation or depression of immune cells in culture can be evoked, depending upon the dosage of the streptococcal membrane preparation used.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $49,969 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune Related Cell Lines

Principal Investigator: Name/Address Performing Organization:	Dr. Melvin Cohn The Salk Institute San Diego, California 92112

Contract Number: N01-CB-23886

Starting Date: 2/26/74 Expiration Date: 2/25/75

Goal: To establish a library of lymphoma and myeloma tumor cell lines and make these available to investigators.

Approach: The contractor will catalog the available cell lines in his laboratory and characterize them as regards their lymphoma and myeloma characteristics. The results will be put into a catalog and either frozen cell lines or mice carrying the appropriate tumors will be made available to investigators on demand.

Progress: A catalog of over 100 culture and tumor cell lines is maintained. Over two hundred shipments of tumor lines or mice bearing tumors were distributed to investigators throughout the world during the last contract year.

Significance for Cancer Research (NCP Objective 6 Approach 1) Experimental cancer immunology studies require cell lines and tumors of known characteristics. This contract provides a national resource which supports cancer research throughout the country.
Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: April 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $89,115 (Est.) $7,000 Supplement Negotiated

CONTRACT RESEARCH SUMMARY

Title: Supramolecular Organization of Normal and Tumor Cell Surfaces and Their Relationship to Escape from Immune Surveillance and Growth Control

Principal Investigator: Name/Address Performing Organization:	Garth L. Nicholson, Ph.D. The Salk Institute for Biological Studies P. O. Box 1809 San Diego, California 92112
Contract Number: N01-CB-33879	
Starting Date: 6/25/73	Expiration Date: 9/74

Goal: To examine quantity and spatial organization of cell surface antigens and saccharides in normal and malignant cells.

Approach: Normal and transformed cells in tissue culture will be reacted with radioisotope labeled antibodies and lectins and then studied with electron microscopic and cell fractionation procedures.

Progress: Dynamics of specific cell surface receptors on normal and neoplastic cells have been investigated using affinity purified plant lectins and antibodies as qualitative, quantitative and ultrastructural probes. Several lectins were purified and characterized in the course of these studies.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Understanding cell surface changes underlies many approaches to cancer prevention.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology
Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $136,634

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells in Tumor Masses

Principal Investigator:	Stephen W. Russell, DVM, Ph.D.
Name/Address	Scripps Clinic and Research Foundation
Performing	La Jolla, California 92037
Organization:	

Contract Number: N01-CB-44001
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To provide information about the numbers of immune-related cells in tumor masses, their identification, function in the tumor mass and factors influencing their movement.

Approach: The contractor will undertake precise identification and quantitation of immune-related cells in neoplasms. An attempt will be made to develop improved methods for use in identification and quantitation of immune-related cells in neoplasms and improved methods of tumor disaggregation.

Progress: Techniques have been developed which permit disaggregation of tumors with high yields of viable cells, and methods have been provided for identification and quantification of immune-related cells in neoplasms.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $90,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: The Use of Recombinant Inbred Strains of Mice for Analysis of Ir-1, Rgv-1 and Closely Linked Genes

Principal Investigator: Hugh O. McDevitt, M.D.
Name/Address Stanford University School of Medicine
Performing Stanford, California
Organization:

Contract Number: N01-CB-43941
Starting Date: 3/1/74 Expiration Date: 2/1/75

Goal: To develop and test mice bearing recombinant H-2 alleles in relation to analysis of the immune response.

Approach: The contractor will develop and maintain large breeding colonies of inbred mice for use in production and testing of antisera directed against a variety of known H-2 recombinants.

Progress: Major problems concerning animal care procedures which were identified early in the contract period have been corrected, and procedures for adequate disease control and genetic monitoring have been instituted.

The sizes of the breeding colonies have been increased, and production of new congenic lines has been initiated.

Significance for Cancer Research (NCP Objective 2 Approach 3)
Studies of genetically influenced immune responses are an important part of the baseline information flow of the Tumor Immunology Contract Program.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Feb. 1974
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $70,323

CONTRACT RESEARCH SUMMARY

Title: Nature of Interactions Between Tumor Cells and Immunoglobulins

Principal Investigator: Name/Address Performing Organization:	Dr. Issac P. Witz Tel Aviv University Tel Aviv, Israel

Contract Number: N01-CB-43868
Starting Date: 8/15/73 Expiration Date: 8/74

Goal: This contractor will examine the role of natural and tumor specific immunoglobulin in coating of tumor cells in relation to antibody mediated and lymphocyte mediated cytotoxicity.

Approach: The contractor will (1) prepare purified Ig from acid eluates of coated tumor cells; (2) examine ascites T lymphoma, mammary carcinoma, and carcinogen-induced sarcoma cells for complement-dependent cytotoxicity, lymphocyte-mediated cytotoxicity and antibody-mediated cytotoxicity, using uncoated tumor cells as model systems; (3) utilize mice who have been immunized with noncross-reactive antigens and then challenged with ascites tumor cells and examine whether these cells show the capacity to bind the radiolabeled immunizing antigens and its effect on cytotoxicity assays; and (4) study the surface binding capacity of cultured tumor cells for various immunoglobulin fractions obtained from normal mice or from plasmacytoma bearing mice.

Progress: Experiments are in progress on eluates of a mouse lymphoma and a mouse mammary carcinoma. It has been established that IgG containing eluates can block complement-mediated cytotoxicity to tumor target cells in at least one system.

Significance for Cancer Research (NCP Objective 3 Approach 4)
The capacity of the immune system to recognize and eliminate transformed cells may depend on and be modified by immunoglobulins.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $47,170

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T-Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Paul H. Maurer Jefferson Medical College of Thomas Jefferson University Philadelphia, Pennsylvania 19107

Contract Number: N01-CB-43999

Starting Date: 5/1/74 Expiration Date: 5/15/75

Goal: To investigate the properties of antigen-binding receptors on antigen-activated T lymphocytes.

Approach: The contractor will study binding of T cells and B cells with cross-reacting immunogens from immune responder mice, immunized non-responder mice and appropriate control non-immunized mice.

Progress: Methodology has been developed for studying binding of synthetic polypeptide antigens to isolated cells and membrane fragments from responder and non-responder mice.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator: Name/Address Performing Organization:	Donald F. H. Wallach, M.D. Tufts - New England Medical Center 171 Harrison Avenue Boston, Massachusetts 02111

Contract Number: N01-CB-44000
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will undertake fractionation and characterization of plasma membranes of normal and neoplastic lymphocytes, using normal and SV40 transformed lymphoid cells as a model. Membrane dynamics will be studied and an attempt will be made to establish the dynamic topology of normal and SV-40 transformed lymphocytes.

Progress: Methods have been developed for preparation of highly purified plasma membranes in high yield from normal and SV40 transformed lymphocytes. Fractionations are being monitored by scanning electron microscopy and use of membrane markers.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $90,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Name/Address Performing Organization:	Paavo Toivanen, M.D. Turku University Medical School Turku, Finland

Contract Number: N01-CB-43971

Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To increase understanding of lymphoid cell differentiation, which is fundamental in investigations of immunologic responses to tumors.

Approach: The contractor will carry out studies on origin and differentiation sites of pre-bursal stem cells in chickens and on mechanisms of T cell differentiation during early ontogeny in man and sheep.

Progress: Preliminary studies have been done on determination of origin and differentiation sites of prebursal stem cells in chickens. Studies of the mechanisms of T cell differentiation during early ontogeny in man and sheep are also underway.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $56,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Control of Granulocyte and Macrophage Proliferation in Leukemia and Related Neoplasma

Principal Investigator: Name/Address Performing Organization:	Donald Metcalf, M.D. The Walter and Eliza Hall Institute of Medical Research Victoria 3050, Australia

Contract Number: N01-CB-33854
Starting Date: 6/20/73 Expiration Date: 10/19/74

Goal: To examine the regulator substance known as colony stimulating factor (CSF) in relation to its formation, mechanisms of action, and associations with cancer.

Approach: 1) Improve methods for producing the regulator substance Colony Stimulating Factor (CSF) and study its mode of action on target cells, both normal and neoplastic, 2) study the mechanisms by which antigens stimulate the formation of CSF, 3) evaluate the role of CSF in the regulation of granulopoiesis and monocyte formation in preleukemic and leukemic patients as well asnormal humans and animals.

Progress: Procedures were successfully developed for producing colony stimulating factor using mouse lung tissue, and CSF production was shown to be stimulated by bacterial antigens. Other CSF studies were carried out in three different model tumor systems, providing evidence suggesting that the CSF-CFC system is involved in host responses to tumor cells.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Humoral factors, such as CSF, which regulate cells of the immune system are potentially powerful tools for immunotherapy.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Deferred
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $127,000

CONTRACT RESEARCH SUMMARY

Title: The Role of T and B Lymphocytes in Tumor Immunity

Principal Investigator: Name/Address Performing Organization:	Dr. G. J. V. Nossal The Walter and Eliza Hall Institute of Medical Research Victoria 3050, Australia

Contract Number: N01-CB-23889
Starting Date: 10/30/74 Expiration Date: 10/29/75

Goal: The work of this project is to analyze the relative roles of T and B cell mediated immune responses in the rejection of tumors in mice.

Approach: The approach will include an analysis of the mechanisms whereby tumor cells are killed and comparisons of various methods of inducing immune response against tumors. Plasma cell tumor systems will be utilized for at least some of the experiments, but fibrosarcomas, spontaneous reticulum cell sarcomas, paraffin oil induced reticulum cell sarcomas, and other experimental tumors will also be studied. All will be examined from the point of view of establishing optimum methods for producing T cell immunity. A portion of this work will require biophysical separation methods that will be applied to permit separate study of T and B lymphocytes. Techniques presently in hand, as well as new procedures that are under investigation, will be applied to the separation of these cells and then the evaluation of the biologic function of the separated cells in tumor systems. The in vitro activation of T lymphocytes and an analysis of their killer activity will be undertaken. In addition, studies will be made of mechanisms for blockading killer cell activity. The final portion of the contract will deal with the mechanism of action of chemically defined adjuvants and their possible role in cancer immunology.

Progress: A large number of transplantable mouse tumors have been screened for capacity to provoke tumor-specific immunity and some have been established as continuous cell lines in tissue culture.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Understanding and manipulation of T and B cell populations are crucial to immunotherapy of cancer.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Waived 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $129,781

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Name/Address Performing Organization:	Sen-itoroh Hakomori, M.D., Ph.D. University of Washington Dept. of Pathobiology Seattle, Washington

Contract Number: NO1-CB-43920
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate and chemically characterize "galactoprotein a" "ceramide X" and sialylgalactosyl or sialylgalactosaminyl protein showing specific label for tumors and prepare, purify and characterize antibodies directed against such surface labelled components.

Progress: The presence of a large externally labelled glycoprotein on non-malignant cell lines and its absence from malignant lines has been studied and studies on somatic hybrids between such lines have been initiated.
A major advance was the isolation and characterization of a unique surface-labelled glycolipid present in transformed hamster cells.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $70,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components
Relevant to Tumor Immunology

Principal Investigator: Name/Address Performing Organization:	Dr. E. D. Kiehn University of Washington Seattle, Washington 98195

Contract Number: N01-CB-43987

Starting Date: Expiration Date:

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will study cell lines transformed by wild type and mutant viruses in comparison to their normal counterparts, investigating regulation of growth levels _in vitro_ and control of cell surface morphology.

Progress: Transformed cells have been shown to become rounded and show increased surface blebbing. Enzymes active on cell surface carbohydrates did not produce such blebbing on normal cells although proteases did.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $28,000

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanisms by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Name/Address Performing Organization:	Dr. Nechama Haran-Ghera Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-43930

Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To determine which mechanisms are important in preventing an immune response from effectively destroying tumor cells.

Approach: The contractor will investigate the mechanisms of escape from controlled proliferation to overt leukemia development in C57 BL/6 mice.

Progress: Progress has been made toward isolation and characterization of pre-leukemic cells. Experiments involving isolation of pre-leukemic cells from spleen colonies *in vitro* are in progress.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $90,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Effect of Thymic Cells and Factors on Tumors

Principal Investigator: Name/Address	Dr. Nathan Trainin Weizmann Institute of Science Rehovot, Israel
Performing Organization	

Contract Number: N01-CB-23890 (Part III)

Starting Date: 6/26/72 Expiration Date: 5/25/75

Goal: To determine whether induction of a hyperthymic state in normal animals affects proliferation of transplanted tumors.

Approach: In thymus studies, a wide range of concentrations of syngeneic thymus cells or crude thymic humoral factors will be tested for ability to alter the proliferation of transplanted tumors.

Progress: Partial inhibition of syngeneic tumor growth in vivo has been achieved by administration of syngeneic lymphocytes sensitized with thymic humoral factor on tumor monolayers.

Significance for Cancer Research (NCP Objective 5,6 Approach 4,1)
Specific cell populations will have to be appropriately manipulated to take maximum advantage of the potentialities of immunotherapy.

Project Officer: Dr. B. H. Sanford, T. Borsos, J. R. Wunderlich, H. J. Rapp
Program: Immunobiology Site Visit Date: August, 1974
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $130,000 (Est.)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Name/Address Performing Organization:	Dr. D. C. Edwards Wellcome Research Laboratories Beckenham, Kent, England

Contract Number: N01-CB-43929
Starting Date: 6/20/74 Expiration Date: 6/19/75

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study means for selectively stimulating T cell responses to well-defined antigens. An attempt will be made to distinguish adjuvants which act primarily on T cells and others which act on B cells and to devise methods for linking these adjuvant molecules to antigens and to antibodies.

Progress: Dr. Zakarian was recruited to work on this contract as proposed and is now engaged 100% of his time on this project. The work done in the first 8 months has been essentially preparative. Substantial progress has been made in developing materials and assays necessary for the work proposed.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $16,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Oliver Smithies University of Wisconsin Madison, Wisconsin 53706

Contract Number: N01-CB-43986
Starting Date: 6/28/74 Expiration Date: 6/27/75

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will work on development of improved methods of determining primary amino acid sequences, suitable for use in sequencing normal and tumor cell surface antigens. β_2 microglobulin and other model proteins will be employed.

Progress: Using β_2 microglobulin as a model, methods were developed for sequencing cell surface antigens. In the course of the work, it was demonstrated that β_2 microglobulin could be labelled with ^{14}C amino acids in small scale tissue culture experiments to a level sufficient to permit sequencing.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $70,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Name/Address Performing Organization:	Dr. Richard K. Gershon Yale University School of Medicine New Haven, Connecticut

Contract Number: N01-CB-43994
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will attempt to identify and characterize subpopulations of lymphocytes with the capacity to suppress responsiveness of other lymphoid cells.

Progress: Evidence has been obtained that suppressor T cells are involved in regulating tumor growth and metastatic spread, and in one system adoptive transfer experiments suggest that the spleens of tumor bearing animals have significant numbers of suppressor T cells.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $71,151 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Chemical Characterization of Purified Thymic Products or Other Agents Promoting Lymphocyte Differentiation

Principal Investigator: Name/Address: Performing Organization:	Dr. Byron H. Waksman Yale University School of Medicine New Haven, Connecticut 06510

Contract Number: N01-CB-43926

Starting Date: 6/16/74 Expiration Date: 11/15/75

Goal: To chemically characterize an agent shown in well defined in vitro assays to promote differentiation of lymphoid cells from precursors.

Approach: The contractor will obtain and purify "lymphocyte activating factor" (LAF) from normal human lymphocytes and test its mitogenic action on mouse thymocytes.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $94,357

CONTRACT RESEARCH SUMMARY

Title: Development of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. Henry Weimer
Name/Address University of California at Los Angeles
Performing Los Angeles, California 90024
Organization:

Contract Number: N01-CB-43860
Starting Date: September 1, 1973 Expiration Date: August 31, 1975

Goal: To identify new tumor associated antigens in carcinomas of the colon, stomach, lung, breast, prostate, and bladder which might be useful for immunodiagnosis.

Approach: Extract antigens from these carcinomas and from comparable normal tissues and from fetal tissues and prepare heterologous antisera against each of the antigens. Antigens shall be purified and antisera rendered specific for tumor associated antigens. Radioimmunoassays shall be developed for the detection of each antigen in serum or urine.

Progress: A large number of samples of malignant, normal and fetal tissues have been obtained at surgery or autopsy and a serum bank with samples from cancer patients, non-cancer patients and normal individuals has been established with partial support by this contract. Established carcinoma cell lines have been grown and used for animal immunization. Cell membranes fractions have been prepared by various methods as a source of tumor-associated antigen. Antisera have been raised in rabbits by immunization with intact cells, cell homogenates and ultracentrifugal gradient fractions from normal, malignant and fetal tissues. Several antisera against colon and prostate tumors showed evidence of antibodies with preferential activity against tumor tissue.

Significance for Cancer Research (NCP Objective 5 Approach 4)
New and more specific immunodiagnostic tests for the common types of human cancer are needed.

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $162,999

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Leonard Chess
Name/Address Performing Organization: Children's Cancer Research Foundation
35 Binney Street
Boston, Massachusetts 02115

Contract Number: N01-CB-43964
Starting Date: 5/16/74 Expiration Date: 5/15/75

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: A variety of cell separation techniques have been developed and/or used including affinity chromatography, E and EAC rosettes, carbonyl iron depletion and nylon wool absorption. Pure populations of human T and B cells, as well as null cells have been isolated. These cell populations have been characterized with respect to cell surface characteristics and _in vitro_ cell mediated immune functions.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Robert K. Oldham, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $79,800 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Improvement of Assays for Cell Mediated Immunity: Methods for Isolation and Preservation of Lymphocytes for Reproducible Reactivity in Immunological Tests

Principal Investigator: Dr. Roy Weiner
Name/Address Performing Organization: Children's Cancer Research Foundation
35 Binney Street
Boston, Massachusetts 02115

Contract Number: N01-CB-43885
Starting Date: 5/16/74 Expiration Date: 5/15/75

Goal: To develop improved techniques for the preservation of the immunological reactivity of human peripheral blood lymphocytes.

Approach: Lymphocytes will be separated from the peripheral blood of normal individuals and of patients with carcinomas and will be preserved in the frozen state and then tested for retention of their biological properties, to include reproducible immunological reactivity.

Progress: Conditions have been defined for optimal recovery of large numbers of cells frozen slowly in DMSO. Reactivity of fresh and cryopreserved cells in functional assays shows virtually total recovery of proliferative responses to mitogen, antigen, and allogeneic cells. It has also been shown that frozen cells were as good as fresh cells as stimulators, responders and targets in the stimulated cytotoxicity assay.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Robert K. Oldham, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $73,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Purified Carcinoembryonic Antigen and Antisera

Principal Investigator: Dr. Charles W. Todd
Name/Address City of Hope National Medical Center
Performing Duarte, California 91010
Organization:

Contract Number: NO1-CB-23877
Starting Date: June 1, 1972 Expiration Date: January 31, 1976

Goal: To provide purified carcinoembryonic antigen and antisera to carcinoembryonic antigen for use in studies involving the immunodiagnosis of cancer.

Approach: The contract will use presently available techniques to purify carcinoembryonic antigen. In addition, the contractor will investigate additional approaches for the preparation and isolation of these materials. Purified antigen will be used to immunize animals and produce antisera of appropriate specificity and strength for use in radioimmunoassay for diagnostic purposes. Purfied CEA will be partially characterized from the chemical point of view, and where indicated, fragments will be prepared and their structure determined.

Progress: The contractor provided 32 other investigators with carcinoembryonic antigen (CEA) and/or antisera and conducted workshops on CEA radioimmunoassays involving 34 trainees. The laboratory has been designated by WHO as a collaborating center for CEA standardization. Advances were made in characterization of the CEA molecule. It was demonstrated that most of the sugars (including 100% of fucose and sialic acid could be removed without decreasing inhibitory activity of the molecule in the radioimmunoassay. Peptide fragmentation studies provided evidence that there is only one peptide chain and that there is more than one antigenic determinant on the CEA molecule. Progress was also made toward solving some of the technical problems involved in obtaining the amino acid sequence of the CEA protein.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Knowledge of the structure of CEA might make possible improved assays for detection of this carcinofetal antigen.

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: Feb. 18, 19, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $108,519

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays for Circulating Tumor Associated Antigens: Clinical Usefulness of Carcinoembryonic Antigen (CEA) Assay as an Adjunct in the Diagnosis of Gastrointestinal Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Theodore Hersh Emory University 1364 Clifton Road, N. E. Atlanta, Georgia 30322

Contract Number: N01-CB-43887
Starting Date: 3/15/74 Expiration Date: 3/14/75

Goal: To determine whether the CEA assay is a useful adjunct in the diagnosis of gastrointestinal cancer.

Approach: Patients who have signs and symptoms suggestive of gastrointestinal cancer will have complete clinical and laboratory evaluation, and in addition CEA values will be determined. Correlation of elevated CEA levels with initial diagnosis of gastrointestinal cancer or development of cancer on subsequent followup will be evaluated.

Progress: A total of 310 patients with one of twelve gastrointestinal signs or symptoms have been enrolled in this study. Approximately 10% have subsequently been proven to have gastrointestinal cancer. Serum and plasma have been obtained on each. Some aliquots are being stored and some have been submitted for CEA determination. The results of these determinations have not been sent to the principal investigator in accordance with the guidelines for this study.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: Feb. 15, 1974
Technical Review Group: Committee om Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $50,200 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays of Circulating Tumor Associated Antigens: Clinical Usefulness of the Alphafetoprotein (AFP) Test and Human Chorionic Gonadotropin (HCG) in the Differential Diagnosis of Testicular Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Charles L. Vogel Emory University 1364 Clifton Road, N. E. Atlanta, Georgia 30322

Contract Number: NO1-CB-43888
Starting Date: 3/15/74 Expiration Date: 3/14/75

Goal: To determine the clinical usefulness of alpha fetoprotein test and test for human chorionic gonadotrophin in the differential diagnosis of testicular cancer.

Approach: Patients with signs or symptoms consistent with the diagnosis of intestinal cancer will be evaluated clinically and will also have AFP and HCG tests performed. Correlation of the test results and diagnosis of cancer will be examined.

Progress: In the seven months of the study to determine the usefulness of AFP and HCG in the differential diagnosis of testicular cancer, 15 previously untreated patients and 14 patients who had previously received treatment have been entered in this study. Serum specimens and pathological slides have been obtained on all 29 patients. Two of the 15 untreated patients with a testicular mass had a testicular cancer. Both had at least one marker elevated. The remaining 13 did not have a testicular carcinoma at operation and had normal serum AFP and HCG levels. It is expected that the principal investigator will be able to expand the number of patient studies over the next year and provide useful information on the value of these markers in diagnosis of testicular tumors and in following the efficacy of therapy.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: Feb. 15, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $22,800 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Dr. Ariel Hollinshead
Name/Address George Washington University
Performing 2300 I Street, N. W.
Organization: Washington, D. C. 20037

Contract Number: NO1-CB-92176
Starting Date: June 27, 1969 Expiration Date: Feb. 28, 1976

Goal: To determine whether skin testing for delayed hypersensitivity reactions can be used to measure cellular immunity to tumor-associated antigens. To use the skin test technique as an assay for separation and purification of tumor antigens.

Approach: Extraction and purification of skin reactive antigens from carcinoma of the breast, malignant melanoma, and acute leukemia.

Perform skin tests on patients at various stages of disease, to determine relationship of reactivity to clinical course.

Provide antigens for possible use in *in vitro* assays of cell-mediated immunity.

Progress: Detailed studies on skin reactive antigens associated with malignant melanoma, carcinoma of the breast and acute leukemia have been done. Two types of skin reactive antigens have been separated in malignant melanoma, including one which seems specific for melanoma. Two distinct antigens have been separated for breast cancer also. Dr. Hollinshead has collaborated with Dr. Wells by providing fetal intestinal skin reactive antigen for testing in a family with familial polyposes. Skin test studies on antigens associated with acute leukemia have also been pursued on a collaborative basis. The contractor has made good progress in partial separation of fractions with different specificities and in establishing specificity of delayed hypersensitivity reactions elicited by her solubilized fractions.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A fundamental problem in tumor immunology is the demonstration of tumor specific antigens in human tumors. This contract provides an opportunity to study delayed skin reactions to antigens.

Project Officer: To be reassigned
Program: Immunology:Immunodiagnosis Site Visit Date: January 3, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $160,000

CONTRACT RESEARCH SUMMARY

Title: Development of Animal Models for Circulating Tumor Associated Antigens Potentially Useful in the Immunodiagnosis of Carcinoma

Principal Investigator: Name/Address Performing Organization:	Dr. James W. Osborne University of Iowa Iowa City, Iowa 52242

Contract Number: NO1-CB-43891
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: Perform studies of circulating tumor associated antigens in animal carcinomas that would serve as a model for common human cancers and that are potentially useful in developing immunodiagnostic tests for cancer.

Approach: Study antigens associated with radiation induced intestinal tumors in mice. Demonstrate and isolate circulating tumor associated antigens and design specific tests for quantitating the antigen. Study the relationship of antigen appearance to pre-malignant lesions and determine the levels of antigen in relation to tumor size.

Progress: A pool of tumor rats bearing recognizable intestinal mucoid adenocarcinomas at various stages of development has been generated for future investigations. Antisera to various cell organelles of neoplastic tissue have been raised and tested. Preliminary investigations show existence of a tumor-associated protein localized at the cellular membranes of adenocarcinoma. The protein has been immunochemically characterized. Progress has been made in development of a sensitive and specific assay for quantitation of circulating tumor-associated antigen(s) in serum samples from rats bearing radiation-induced intestinal tumors.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Chou-Chik Ting, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $88,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Improvements of Assays for Cell Mediated Immunity: Development of New *In Vitro* Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing 725 N. Wolfe Street
Organization: Baltimore, Maryland 21205

Contract Number: N01-CB-43965
Starting Date: 5/16/74 Expiration Date: 5/15/75

Goal: Development of new *in vitro* techniques or substantial modifications of existing techniques for the evaluation of cell-mediated immunity to whole tumor cells.

Approach: In syngeneic animal tumor model systems, the use of ^{86}Rb will be compared with the release of ^{51}Chromium for measuring cytotoxicity of tumor cells by immune lymphocytes. The use of gluteraldehyde-fixed tumor cells for lymphocyte stimulation will be examined. The conjugation of soluble tumor antigens to sensitive indicator cells for measurement of cell-mediated immunity to tumor antigens will be examined.

Progress: Work has been done on development of a cytolytic assay system using methylcholanthrene-induced fibrosarcoma as antigen and target cell, and splenic lymphocytes from tumor-bearing syngeneic mice as effector cells because this tumor is promising in several areas of this contract's scope. Much information about the life history of the tumor has been gathered and various cultural and experimental parameters have been developed regarding this tumor. Soluble extracts of the original tumor and tumors subjected to one passage have been made and the testing of such material in tumor bearing hosts is in progress.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: 81,500 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Experimental Systems for Carcinoembryonic Antigen

Principal Investigator: Name/Address Performing Organization:	Dr. David M. Goldenberg University of Kentucky Lexington, Kentucky

Contract Number: N01-CB-33869 Expiration Date: March 31, 1975
Starting Date: June 30, 1973

Goal: To produce carcinoembryonic antigen (CEA) which will then be provided to other investigators for analysis of its structure and to provide cell lines which produce CEA for use in studies of cellular hypersensitivity in patients.

Approach: Will develop two human colon tumor lines maintained in hamster cheek pouches for the purpose of providing carcinoembryonic antigens in quantity to other investigators for analysis of the structure of the material. Will provide cell lines derived from the tumors carried in hamster cheek pouches for the purpose of studying cellular hypersensitivity in patients with various carcinomas.

Progress: Human tumor material from GW-39 tumors has been supplied to seven laboratories. Species specificity and carcinoembryonic antigen content of numerous GW-39 tumor cell lines have been determined as well as their malignant potential in hamsters. Various other cell lines have been propagated and characterized. A high molecular weight CEA has been isolated from GW-39 tumor tissue and chemical characterization has been done on it. The identity of GW-39 CEA to reference human CEA has been established via radioimmunoassay and immunodiffusion.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Structural analysis of CEA from various sources will improve understanding of its relationship to tumor origin and size.

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: None
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $10,900 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Identification and Purification of Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins

Principal Investigator:	Akira Kobata, Ph.D.
Name/Address	Kobe University
Performing	Ikuta-ku
Organization:	Kobe, Japan

Contract Number: N01-CB-43886
Starting Date: In negotiation   Expiration Date:

Goal: To define the nature of the antigenic determinants of carcinoembryonic antigen and other cellular glycoproteins.

Approach: Develop enzymatic methods for obtaining oligosaccharides with serological activity. Purify and characterize endoglycosidases that will act on CEA, blood group glycoproteins or other macromolecules containing carbohydrate antigenic determinants.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer:
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $87,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Study of Detection of Carcinoembryonic Antigen in Humans

Principal Investigator: Dr. Calvin A. Saravis
Name/Address Mallory Institute of Pathology
Performing Boston, Massachusetts 02118
Organization:

Contract Number: N01-CB-33264
Starting Date: June 1, 1973 Expiration Date: Nov. 30, 1975

Goal: Investigate colonic tumor-associated antigens.

Approach: Isolate and characterize colonic tumor associated antigens, produce antisera to the antigens, by using tumor tissue fractions and tumor cell membranes, detect tumor-associated enzyme activity.

Progress: The solid Phase radioimmunoelectrophoresis radio assay was modified to sensitivity of 5ng CEA/ml, and reproducibly measures nanogram quantities of CEA per ml of serum. A counterimmunoelectrophoresis system with consistent coupling of antibody to affinity chromatography beads with negligible variation in the amount of ^{125}I-CEA bound was developed. The contractor has produced antisera to whole tumor extracts in mature and tolerant rabbits, and to specific tumor antigens isolated by newer fractionation procedures. Two rabbits rendered tolerant to corresponding normal tissue have demonstrated antibodies to a component present in primary and metastatic tumors when injected with a zinc glycinate preparation of metastatic tumor. This component is not present in the corresponding normal tissue; it is distinct from CEA, alpha-fetoprotein and ABO or Lewis blood group antigens; and therefore, it represents a possible new tumor associated antigen which was detected in 5 of 13 colonic cancers tested.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $115,073

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens of the Gastrointestinal Tract Other Than Carcinoembryonic Antigen and Alphafetoprotein

Principal Investigator: Dr. Elliot Alpert
Name/Address Performing Organization: Massachusetts General Hospital
Boston, Massachusetts 02114

Contract Number: N01-CB-43889
Starting Date: 6/01/74 Expiration Date: 5/31/75

Goal: To search for new tumor associated antigens of the gastrointestinal tract, which may be more useful for early diagnosis than CEA or AFP.

Approach: Identify by immunological methods antigens which may be useful in the detection of carcinoma of the gastrointestinal tract. Employ these methods in screening both biopsy specimens and cell cultures of gastrointestinal tract tumors for new antigens. Characterize new antigens and demonstrate usefulness for large scale testing of patient populations.

Progress: This is a joint collaborative project between Alpert and Schur, although the tasks and responsibilities are explicitly divided between the two investigators according to their areas of expertise and facilities and they have separate budgets. The progress can be summarized on the joint effort:

Tissues have been collected and extractions of soluble cytoplasmic antigens and cell membrane antigens were made. Techniques for preparation of isolated cells from solid organs have been developed and modified. Sera from patients with histologically documented carcinomas of various types have been collected, as well as sera from patients with benign inflammatory diseases. Partially tolerant rabbits were immunized with various carcinoma extracts; antisera were raised and absorbed; antisera were precipitin tested and tested by immunofluorescence. Some antisera have demonstrated promising results; for example, work is in progress to determine whether or not one liver tumor extract antisera pool is recognizing AFP or a new liver cell carcinoma antigen.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: R. Raymond Gantt, M.D.
Program: Immunodiagnosis Site Visit Date: April 5, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $58,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Blood Sample Collection Bank as Reservoir for Evaluation of Cancer Detection Tests

Principal Investigator: Dr. Heinz Wahner
Name/Address Mayo Foundation
Performing 200 First Street, S. W.
Organization: Rochester, Minnesota 55901

Contract Number: N01-CB-23879
Starting Date: June 15, 1972 Expiration Date: 11/14/75

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the tumor immunology-immunodiagnosis program.

Progress: During the first 24 months blood samples from 4548 patients have been collected. Ten types of G.I. tumors and many other categories of malignant tumors are covered. Sera from several benign tumors and many non-malignant diseases, as well as smokers and normal healthy subjects are represented in the plasma bank. Thousands of vials have been shipped to various investigators.

Significance for Cancer Research (NCP Objective 5 Approach 4)
This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: To be reassigned Site Visit Date: Feb. 19, 1974
Program: Immunology:Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $111,500

CONTRACT RESEARCH SUMMARY

Title: Immunodiagnostic Testing, Antibody Measurement and Fractionation of Serum and Urine

Principal Investigator: Roy Woods, Ph.D.
Name/Address Performing Organization: Meloy Laboratories, Inc.
Springfield, Virginia 22151

Contract Number: N01-CB-33875
Starting Date: November 26, 1965 Expiration Date: January 10, 1975

Goal: To use quantitative and qualitative immunochemical techniques for improved diagnosis of cancer and to evaluate humoral immune responsiveness of patients with cancer.

Approach: Qualitative and quantitative immunochemical techniques are used for the diagnosis of multiple myeloma, Waldenstrom's macroglobulinemia, agammaglobulinemia, and other diseases with immunoglobulin disorders. In addition, these same techniques are used to evaluate carcinoembryonic antigen and α-fetoprotein in the serum of patients with a variety of malignancies. Studies are carried out to classify the abnormal proteins of the abnormality diseases and to study the α-fetoprotein and carcinoembryonic antigens in relation to diagnosis, course of disease, and response to therapy. Appropriate reagents for the immunoglobulin and carcino-fetal antigen detections are prepared and evaluated. In addition, the Immunoglobulin Reference Center serves as a distribution outlet for provision of reference material for human serum immunoglobulin quantitation to qualified laboratories and in cooperation with the World Health Organization prepares and distributes international immunoglobulin reference serums. The Immunoglobulin Reference Center will also serve as a distribution point for carcino-fetal standard preparations when they are available. Finally, the Reference Center serves as a focus for the education of investigators and their technicians in the techniques and interpretations of various immunodiagnostic procedures.

Progress: CEA assays have been used to follow patients with metastatic disease during drug therapy. Preliminary data indicate that CEA fluctuates with, but does not precede, other measures of tumor load in patients with advanced cancer. Alphafetoprotein and human chorionic gonadotrophin levels are also being determined on serums from patients with cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after therapy.

Project Officer: W. D. Terry, M.D., M. Blaese, M.D., T. A. Waldmann, M.D.
Program: Immunodiagnosis Site Visit Date: 11/19/73
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY 74 Funds: $340,223

CONTRACT RESEARCH SUMMARY

Title: Procurement of Tumor Specimens and Serial Serum Specimens from Cancer Patients

Principal Investigator: Eugene B. Rosenberg, M.D.
Name/Address Performing Organization: University of Miami
Coral Gables, Florida 33124

Contract Number: N01-CB-43861
Starting Date: August 15, 1973 Expiration Date: 10/31/75

Goal: Collection of serum specimens from patients with malignant diseases over a period of time to be used for the development and evaluation of potential immunodiagnostic tests for cancer.

Approach: Serum and plasma specimens will be obtained from patients with carcinomas of the colon, lung, breast, prostate, bladder, and other malignant diseases and from patients with benign diseases. A minimum of 25 patients per year of each category will be obtained. Specimens will be obtained before any therapy and at weekly intervals for one month and every two to three months thereafter.

Surgical specimens of the above tumors to the extent of a minimun of 25 tumors of each type per year will be obtained under sterile conditions and in conjunction with normal control tissues from the same organ sites. Serum, EDTA, plasma, and heparinized blood from some patients may also be required. In addition, a certain amount of dissection of tumor tissue and preparation of single cell suspensions, with appropriate packaging for shipping fresh and frozen specimens will be done.

Progress: A large variety and number of tumor specimens have been collected and subsequently distributed to five designated centers throughout the country. Serum samples from patients with a wide variety of cancers have also been collected. Detailed clinical information has been provided with specimens when they are distributed.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Readily available collection of sera obtained from patients during the course of therapy for their cancers are necessary for prompt evaluation of promising new immunodiagnostic techniques.

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $155,700

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Name/Address Performing Organization:	John S. Najarian, M.D. University of Minnesota Minneapolis, Minnesota 55455

Contract Number: NO1-CB-33915
Starting Date: June 30, 1973 Expiration Date: 4/30/75

Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on an annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: During the past year 2405 asymptomatic adults in the high cancer risk age (50-70 years) were examined for malignancy. Histories and physicals were done. Serum was collected at the examination and aliquoted and frozen for shipment to the regional storage center at the Mayo Clinic.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: To be reassigned.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $78,255 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Assays for Immune Depression in Cancer Patients

Principal Investigator: Edmond J. Yunis, M.D.
Name/Address Performing Organization: University of Minnesota
Minneapolis, Minnesota 55455

Contract Number: N01-CB-43853
Starting Date: August 1, 1973 Expiration Date: 7/31/75

Goal: To develop quantitative assays for the depression of various aspects of the immune response in human cancer patients and for following the clinical course of cancer patients.

Approach: Various parameters of the immune response will be evaluated in patients having carcinomas of the lung, breast, colon, prostate and bladder. These assays will be potentially applicable to large-scale use and will have been previously shown to be depressed in some cancer patients or to be depressed in animals with experimental tumors.

Progress: Patients with basal cell cancer and various carcinomas were tested for the percent of T and B cells in peripheral blood, and for the ability of their lymphocytes to be stimulated by mitogens, antigens and allogeneic leukocytes. It was found that the cancer patients as a group had significant depression in stimulation by some mitogen doses and by some antigens. The total T and B cells in the peripheral blood of cancer patients was generally the same as that of the normal controls.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Assays for evaluation of the immune responsiveness of given individuals may prove to be highly important in understanding cancer patients' susceptibility to their tumors.

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: May 10, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $117,366

CONTRACT RESEARCH SUMMARY

Title: Detection by Immunofluorescent Techniques of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Fiorenzo Paronetto Mt. Sinai School of Medicine 5th Avenue at 100th Street New York, New York 10029

Contract Number: NO1-CB-43953
Starting Date: 5/16/74 Expiration Date: 5/15/75

Goal: To identify specific antibodies in the sera of patients with carcinoma of the lung, breast, and colon.

Approach: Sera from cancer patients and from controls will be tested against autologous tumor cells by immunofluorescence assays with viable and fixed tumor cells. Sera for testing will be obtained before and at appropriate intervals after therapy. Specificity of observed reactions will be determined.

Progress: Specimens of tumors and autologous sera from patients with colon or lung tumors or with breast disease have been collected and processed. Sera from patients with several types of benign and malignant tumors were screened using cryostat sections from a breast adenocarcinoma and a breast fibroadenoma. Cell cultures from the three types of tumors have been started and some have been subcultured; karyotype of the cell lines is being determined.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $82,830 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Henry Altschuler, Ph.D.
Name/Address Philadelphia Geriatric Center
Performing 5301 Old York Road
Organization: Philadelphia, Pennsylvania 19141

Contract Number: NO1-CB-33914
Starting Date: June 25, 1973 Expiration Date: 10/24/75

Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on an annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: In the first period of this contract over 700 sera specimens have been collected and a log book on the subjects has been kept. Separate files are kept on the persons who have died, with autopsy findings when available, and a cancer file index is kept on those who have developed malignancies. In addition to the above information, forms used in this study contain information concerning asthma, allergy, rheumatoid arthritis, desensitization therapy, immuno-suppressive therapy, immunization, blood transfusion and date of last physical exam.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $44,665

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens of the Gastrointestinal Tract Other Than Carcinoembryonic Antigen and Alphafetoprotein

Principal Investigator: Dr. Peter H. Schur
Name/Address Robert B. Brigham Hospital
Performing Boston, Massachusetts 02120
Organization:

Contract Number: N01-CB-43858
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To search for new tumor associated antigens of the gastrointestinal tract, which may be more useful for early diagnosis than CEA or AFP.

Approach: Identify by immunological methods antigens which may be useful in the detection of carcinoma of the gastrointestinal tract. Employ these methods in screening both biopsy specimens and cell cultures of gastrointestinal tract tumors for new antigens. Characterize new antigens and demonstrate usefulness for large scale testing of patient populations.

Progress: This is a joint collaborative project between Alpert and Schur, although the tasks and responsibilities are explicitly divided between the two investigators according to their areas of expertise and facilities and they have separate budgets. The progress can be summarized on the joint effort:

Tissues have been collected and extractions of soluble cytoplasmic antigens and cell membrane antigens were made. Techniques for preparation of isolated cells from solid organs have been developed and modified. Sera from patients with histologically documented carcinomas of various types have been collected, as well as sera from patients with benign inflammatory diseases. Partially tolerant rabbits were immunized with various carcinoma extracts; antisera were raised and absorbed; antisera were precipitin tested and tested by immunofluorescence. Some antisera have demonstrated promising results; for example work is in progress to determine whether or not one liver tumor extract antisera pool is recognizing AFP or a new liver cell carcinoma antigen.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: R. Raymond Gantt, M.D.
Program: Immunodiagnosis Site Visit Date: April 5, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $50,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen and Related Tumor-Specific Antigens in the Diagnosis of Cancer and as an Adjuvant in the Management of the Cancer Patient.

Principal Investigator: Name/Address Performing Organization:	Dr. Tsann Ming Chu Roswell Park Memorial Institute 666 Elm Street Buffalo, New York 14203

Contract Number: N01-CB-33858
Starting Date: June 25, 1973 Expiration Date: 10/24/75

Goal: To evaluate the usefulness of carcinoembryonic antigen (CEA) assay in several varieties of human cancer. It will be evaluated for its capacity to 1)monitor recurrence of disease and 2) to measure the effectiveness of therapy.

Approach: This will be done by 1) determining a baseline value on all new patients who may fit into one of these categories, 2) evaluating CEA levels at 4 and 14 days after surgery (with some selected patients having more assays), 3) additional testing will be done at 30 and 60 days and every three months thereafter. For patients receiving radition therapy, CEA testing will be done at two-week intervals during the therapy and at monthly intervals thereafter. Patients on chemotherapy will be tested at weekly intervals for ten weeks and then months.

Progress: During the past year a large number of patients with cancers of the GI tract have been entered in the study and a large number of serial determinations of carcinoembryonic antigens have been performed. Some attempts have been made to correlate the clinical information from the patients with the test data. However, these attempts have not been performed in sufficient depth to allow proper analysis of the use of CEA assays for monitoring cancer patients. A series of changes in the methods for accumulating the clinical data and the analysis and correlations have been made.

Significance for Cancer Research (NCP Objective 5 Approach 4)
CEA is one of the more promising assays for evaluating effects of therapy on certain cancers.

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: None
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $58,474

CONTRACT RESEARCH SUMMARY

TITLE: Isolation and Characterization of Soluble Human Tumor (CEA) Specific Antigens

Principal Investigator: Dr. Ralph Reisfeld
Name/Address Scripps Clinic and Research Foundation
Performing La Jolla, California 92037
Organization:

Contract Number: N01-CP-43277
Starting Date: January 1, 1974 Expiration Date: Dec. 31, 1975

Goal: To isolate and characterize soluble human tumor (carcinoembryonic antigen) specific antigens.

Approach: Isolation of CEA by mild extraction procedures, purification and characterization of CEA, production of anti-CEA antisera and development of an isotope release cytotoxicity assay with CEA positive cell lines.

Progress: It has been shown that CNBr-activation is important in the production of sepharose-based solid phase reactants. A semi-automated immunoadsorbent-based double antibody radioimmunoassay has been developed using a sepharose-bound second antibody and a Biomedical Research Insitute Cell Harvester for processing samples. A specific adsorption method has been developed for isolation of wheat germ lectin, which will probably be useful in CEA purification. A semi-pure CEA has been obtained by gentle isolation and it seems to be considerably more homogenous than does CEA prepared by the perchloric acid precipitation procedure. The contractor has been able to remove metastatic lesions of G.I. tumors using a sepharose-bound anti-CEA immunoadsorbent. The bound CEA injected into rabbits produced IgG capable of binding ^{125}I CEA.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $94,396

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Jorgen E. Fogh, M.D.
Name/Address Performing Organization: Sloan-Kettering Institute for Cancer Research
145 Boston Post Road
Rye, New York 10580

Contract Number: N01-CB-43854
Starting Date: August 8, 1973 Expiration Date: 8/7/75

Goal: To provide human tumor materials needed for the performance of assays for cell-mediated immunity.

Approach: Tumor specimens from patients having the more common tumors, such as carcinomas of the colon, stomach, lung, breast, prostate, and bladder, will be obtained from local sources and utilized for the development of techniques suitable for the isolation of viable tumor cells in good yield separated from contaminating normal cells. Tissue culture techniques which favor the maintenance of high viability of tumor cells will be developed. Cells maintained in such culture will be characterized by morphology and as many other techniques as possible to document their tumor origin. Techniques will be developed for the slow freezing and retrieval of viable cells suitable for growth in tissue culture or for immediate use in immunologic assays.

Progress: 156 cell lines derived from human tumors have been collected. These include at least five lines in each of these categories: breast, lung, colon, brain, kidney and melanoma. Cell characterization is in progress; tests for the presence of mycoplasma are done. In vitro cytopathology diagnosis or chromosome data are available on a large number of these lines. All established lines are being frozen and a computerized catalog of cell characterization is continually updated. Early cell culture work is in progress including tumors of special interest to the program.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Studies of cell-mediated immunity towards specific tumors as well as a general phenomenon in cancer patients is an urgently needed area of development in tumor immunology.

Project Officer: To be reassigned
Program: Immunodiagnosis Site Visit Date: October 31, 1973
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: 129,742

CONTRACT RESEARCH SUMMARY

Title: Detection by Immunofluorescent Techniques of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Name/Address Performing Organization:	Dr. George J. Friou University of Southern California 2025 Zonal Avenue Los Angeles, California 90033

Contract Number: N01-CB-43881
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: To identify specific antibodies in the sera of patients with carcinoma of the lung, breast, and colon.

Approach: Sera from cancer patients and from controls will be tested against autologous tumor cells by immunofluorescence assays with viable and fixed tumor cells. Sera for testing will be obtained before and at appropriate intervals after therapy. Specificity of observed reactions will be determined.

Progress: A systematic method of recruiting patients, collecting specimens and following patients after surgery for breast, lung or colon carcinoma has been instituted in five hospitals. Sera from a large number of patients have been collected and tumor material has been gathered from patients that actually became enrolled in the study. An indirect immunofluorescent test for serum antibodies against cytoplasmic and membrane antigens of tumor cells has been adopted after evaluation of a number of technical alternatives. The effect of chemical fixatives on cell substrates has been examined. Reactions with surface membranes and cytoplasmic antigens were tested using autologous and allogeneic tumor cells. Sera from 39 breast cancer patients were tested for reactivity to allogeneic tumor cells and there was a suggestion of preferential reactivity of antibody to breast cancer cells. Studies regarding specificity via absorption tests are in preliminary stages. Overall, the studies suggest that antibodies to tumor associated antigens are detectable in patients with breast, colon and lung cancer.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $74,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Identification and Purification of Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins

Principal Investigator: Dr. Om Bahl
Name/Address State University of New York
Performing Buffalo, New York 14214
Organization:

Contract Number: N01-CB-43989
Starting Date: 5/1/74 Expiration Date: 4/30/75

Goal: To define the nature of the antigenic determinants of carcinoembryonic antigen and other cellular glycoproteins.

Approach: Develop enzymatic methods for obtaining oligosaccharides with serological activity. Purify and characterize endoglycosidases that will act on CEA, blood group glycoproteins or other macromolecules containing carbohydrate antigenic determinants.

Progress: Various substrates such α_1-acid glycoprotein, fetuin, and porcine submaxillary mucin and their glycopeptides were prepared. A neuraminidase inhibitor, N-(p -aminophenyl)-oxamic acid was synthesized. The assay procedure for endoglycosidases was further developed and optimal growth conditions for various microorganisms were worked out. A number of these microorganisms were examined for endoglycosidase activity.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $53,800 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Improvements of Assays for Cell Mediated Immunity: Development of New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. John T. Harrington
Name/Address University of Texas Medical School
Performing San Antonio, Texas 78284
Organization:

Contract Number: NO1-CB-43893
Starting Date: 6/1/74 Expiration Date: 10/31/75

Goal: To develop new in vitro techniques or substantial modifications of existing techniques for the evaluation of cell-mediated immunity to whole tumor cells.

Approach: Develop Agarose droplet modification of migration inhibition assay for application to detection of immunity to human tumor antigens.

Progress: New Contract; no progress report received yet.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 and FY75 Funds: $97,787

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Hans Wigzell
Name/Address University of Uppsala
Performing Uppsala, Sweden
Organization:

Contract Number: NO1-CB-43883
Starting Date: 6/25/74 Expiration Date: 6/24/75

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: New contract; no progress report received as yet.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: To be reassigned
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $33,500 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens in Carcinoma of the Lung

Principal Investigator: Dr. Robert W. Veltri
Name/Address West Virginia University
Performing Morgantown, West Virginia 26506
Organization:

Contract Number: NO1-CB-43890
Starting Date: 5/16/74 Expiration Date: 5/15/75

Goal: Search for new tumor associated antigens in carcinoma of the lung, which may be more useful in immunodiagnosis than currently available markers.

Approach: Identify by immunological methods antigens which may be useful in detection of carcinoma of the lung. Employ these methods in screening both biopsy specimens and cell cultures of lung tumors, characterize detected antigens and determine distribution of any new antigen or related antibody in patients with cancer, benign disease, and in mormal controls.

Progress Emphasis has been placed on storage of tumors, tumor extracts and tissue cultures in conjunction with continuing work being done on characterization of tumors as they are received. Extraction protocols have been established. Disc and slab polyacrylamide gel electrophoreses have been done on soluble extracts, and the first set of tumor extracts has been injected into rabbits. Several immunoassay procedures have been developed and are being used.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: To be reassigned Site Visit Date: March 15, 1974
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $112,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Development of Animal Models: Early Detection of Antibodies to Carcinomas in Experimental Animals as a Model for Human Studies

Principal Investigator: Dr. Lionel Manson
Name/Address The Wistar Institute
Performing 36th Street at Spruce
Organization: Philadelphia, Pennsylvania 19104

Contract Number: N01-CB-43882
Starting Date: 6/1/74 Expiration Date: 5/31/75

Goal: Development of experimental animal systems for the early detection of antibody response to tumor associated antigens, as model for problem of antibody detection in patients with carcinomas and other malignant diseases.

Approach: Studies will be performed with primary, autochthonous carcinomas or with carcinomas in early transplant, in inbred strains of mice. A sensitive antiglobulin assay will be used to determine antibody levels.

Progress: A spontaneous mammary adenocarcinoma, A-10 line, has been used as a model for present studies and growth conditions have been established. This line is lethal in the syngeneic host at 10 cells animal in less than 50 days. Immunity to low challenge doses of the tumor has been apparently induced with mitomycin-treated cells. Sera have been collected from immunized, normal and tumor bearers for assay for antibodies to the tumor. Preliminary experiments have been done using cultured cells in the radioimmunoassay for antibody against cell surface determinants. Cells treated with proteolytic enzymes have decreased capacity to bind antibody. In normal A mice there are serum antibodies that bind to A-10 cultured cells and the specificity of these antibodies is being examined. Purification procedures to isolate purified surface membrane preparations of the A-10 line are being developed.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: R. Michael Blaese, M.D.
Program: Immunodiagnosis Site Visit Date: Feb. 26, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: 82,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry and Diagnostic Use of Human Tumor Antigens

Principal Investigator: Dr. Vincent Marchesi
Name/Address Yale University
Performing New Haven, Connecticut
Organization:

Contract Number: N01-CB-43859
Starting Date: August 8, 1973 Expiration Date: 11/7/75

Goal: To isolate and purify glycoprotein antigens from human colonic tumors.

Approach: The contract will use various extraction methods to isolate glycoproteins from human colonic tumors and prepare specific antisera to each of the isolated antigens. The specificity of these antigens will be compared with carcinoembryonic antigen prepared by usual procedures. Glycoproteins extracted from primary colon tumors, metastatic tumors, serum, and from embryonic tissues will be examined.

Progress: A lectin-sepharose affinity chromatography system with high capacity has been developed which will enable the contractor to develop large scale isolation procedures. These affinity systems, are very effective for the binding the CEA-like material as isolated from colonic carcinoma patients. Conditions for the adsorption of glycoproteins and CEA-like materials to the columns and for removal of the glycoproteins have been determined. Work has been done on refining conditions for the isolation of membrane glycoproteins which will minimize degradation of sugar, protein and antigenic moieties. All but one of the aldonolactone derivatives needed to inhibit the glycosidases active against the linkages the contractor expects to find in membrane glycoproteins have been synthesized.

Significance for Cancer Research (NCP Objective 5 Approach 4)
New methods for extraction of glycoprotein antigens from human colonic tumors may provide reagents with better specificity for immunodiagnosis than CEA.

Project Officer: J. Frederic Mushinski, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $84,719

CONTRACT RESEARCH SUMMARY

Title: Irradiate Experimental Animals

Principal Investigator: Name/Address Performing Organization:	Dr. William Kiker Armed Forces Radiobiological Research Institute Bethesda, Maryland 20014

Contract Number: NCI-GLC(70)-53
Starting Date: April 6, 1970 Expiration Date: June 30, 1975

Goal: To lethally radiate rhesus monkeys in preparation for bone marrow transplantation in rhesus monkeys and to study and modify the graft versus host reaction (GVHR), the major complication of this form of transplantation.

Approach: Rhesus monkeys receive X-radiation delivered by a Maxitron X-ray generator, in dosages requested by the Project Officer.

Progress: The contractor has satisfactorily radiated rhesus monkeys at our request.

Significance for Cancer Research (NCP Objective 6, Approach 1)
Bone marrow transplantation may allow more intensive cancer chemotherapy than is presently available. Transplantation of allogeneic immune competent cells (bone marrow is one category) may be important in cancer immunotherapy.

Project Officer: G. Nicholas Rogentine, Jr., M.D.
Program: Immunology, Support Site Visit Date:
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY75 Funds: $2500

CONTRACT RESEARCH SUMMARY

Title: Subcellular Fractions in Immunotherapy-Augmentation of Tumor-Specific Immunity with Ribonucleic Acid (RNA)

Principal Investigator: Peter J. Deckers, M.D.
Name/Address Department of Surgery
Performing Boston University School of Medicine
Organization: 80 East Concord Street
Boston, Massachusetts 02118
Contract Number: N01-CB-43949
Starting Date: 06/01/74 Expiration Date: 05/31/75
Goal: Development of a reliable in vivo immunotherapy model with immune RNA in laboratory animals.

Approach: Analysis of the variables associated with optimal extraction and administration of RNA will be studied in small animal models.

Progress: Three relatively large experiments have been performed in the initial contract period. One failed because of changes in the tumor behavior with regard to its antigenicity. The other two experiments, which used another tumor, showed a moderate delay in tumor appearance. This work will be expanded to determine optimal time of harvest of the xenogeneic lymphoid tissues used to extract the immune RNA; concentration of immune RNA for incubating syngeneic lymphoid cells; optimal route of administration of the treated cells; and optimization of other parameters of this complex system. The contractor has successfully initiated a new laboratory in a highly complex field and is now prepared to make significant contributions as to the efficacy of immune RNA in tumor immunotherapy models.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4) Subcellular fractions such as immune RNA may be relatively nontoxic and possibly highly specific approaches to cancer immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $62,186

CONTRACT RESEARCH SUMMARY

Title: Simple Light Microscope Test for B and T Cells on Dried Blood Films (Immunotherapy)

Principal Investigator: William J. Dreyer, Ph.D.
Name/Address
Performing
Organization:
California Institute of Technology
c/o Jet Propulsion Laboratory
4800 Oak Grove Boulevard
Pasadena, California 91109

Contract Number: N01-CB-43875
Starting Date: 03/01/75 Expiration Date: 11/30/75
Goal: Development of a simple histochemical assay for B and T cells.

Approach: Utilizing antibodies specific for mouse B and T cells, the investigator will develop immunolatex reagents specifically for use in conjunction with standard Romanovsky stains. Once the model system has been developed in mice, a human antiserum will be utilized for the development of readily applicable simplified B and T cell assay.

Progress: The contractor has successfully labelled murine B-cells and thymocytes with latex particles coated with anti-sera as evaluated by scanning electron microscopy. In addition, information on the necessary design of non-aggregating and bio-compatible latex particles for use in light microscopy has been developed. The ultimate goal of this project is to apply the technology being developed to the study of human T and B lymphocytes.

Significance for Cancer Research (NCP Objective 4,6 Approach 1,2,4)
Widespread availability of simplified B and T cell assays would be extremely valuable for monitoring immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $90,000

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy of Patients without Detectable Disease after Removal of Tumor-containing Lymph Nodes

Principal Investigator: Donald L. Morton, M.D.
Name/Address Department of Surgery
Performing University of California-Los Angeles
Organization: Los Angeles, California 90024

Contract Number: N01-CB-43852
Starting Date: 08/08/74 Expiration Date: 08/07/75
Goal: Conduct a suitably randomized study to evaluate promising preliminary evidence that BCG therapy is useful in melanoma.

Approach: Patients with a diagnosis of melanoma who have proven involvement of regional lymph nodes and whose disease has been totally removed as far as can be determined are appropriately randomized into treatment groups with due regard for sex, age, and disease classification. The three treatment arms are: 1) no further therapy; 2) BCG by tine injection; and 3) BCG and melanoma cells intradermally. Clinical studies necessary to establish the diagnosis and document the patient's general health status, tumor status, toxicity of therapy, time to relapse, and survival time are being made. Some patients' immunologic status is being followed with a variety of *in vitro* assays.

Progress: The number of patients being entered into the protocol is outstanding. Presently, an average of eight patients per month are being entered into the study, leading to projections that there will be a total of 180-200 patients by the end of the second contract period. It will be entirely satisfactory to the Tumor Immunology Program for them to continue at approximately the same level of effort with some increase for increasing costs. The level of effort as revealed at the time of the site visit has been most variable in the area of the immunologic tests performed. It is the intent of the program that the contract will continue to perform purification and freezing lymphocytes, recall skin testing battery, and blood processing for human serum on all patients taken into the protocol and to perform other assays only selectively (complement fixation; preparation of allogeneic vaccine; lymphocyte toxicity; lymphocyte blastogenesis, MLC and rosette; immune adherence; and soluble antigen preparation for skin test).

Significance for Cancer Research (NCP Objective 6 Approach 4)
This is a direct extension of important preliminary observations regarding immunotherapy in human melanoma. This contract will permit a suitably controlled, randomized clinical trial which should resolve a number of questions about this type of immunotherapy.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: March 10, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $219,822

CONTRACT RESEARCH SUMMARY

Title: Hybrid Tumor Cells: An Immunotherapeutic Agent

Principal Investigator: Robertson Parkman, M.D.
Name/Address Children's Hospital Medical Center
Performing 300 Longwood Avenue
Organization: Boston, Massachusetts 02115

Contract Number: N01-CB-43876
Starting Date: 05/16/74 Expiration Date: 05/15/75
Goal: Evaluate hybrid cells formed by fusion of tumors with non-tumor cells as immunotherapeutic agents.

Approach: Syngeneic animal systems will be used to evaluate the capacity of cells formed by fusion of tumor cells with fibroblasts to alter morbidity and mortality of tumor-bearing animals. In vitro comparison of immune response to hybrid tumor cells, irradiated tumor cells and live tumor cells in normal, tumor-bearing, and "carrier" immunized animals will be evaluated.

Progress: The contractor has achieved fusion of C_3H fibroblasts with a methylcholanthrene induced fibrosarcoma after deriving drug-resistant mutants of the tumor. Similar fusion has been achieved with B-16 melanoma. These hybrid tumor cells have been shown to be capable of immunizing host animals against subsequent lethal tumor challenge. Early results in immunotherapeutic experiments have shown one hybrid cell line to be effective when used 14 days after tumor challenge.

Significance for Cancer Research (NCP Objective 2,6Approach 1,4)
This project will explore a promising new direction in cancer therapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Extimated $32,000

CONTRACT RESEARCH SUMMARY

Title: Human Lung Cancer - Evaluation of BCG Therapy

Principal Investigator: Robert H. Waldman, M.D.
Name/Address University of Florida College of Medicine
Performing c/o J. Hillis Miller Health Center
Organization: Gainesville, Florida 32601

Contract Number: N01-CB-43857
Starting Date: 11/15/74 Expiration Date: 11/14/75
Goal: Evaluate immunotherapy in cancer patients with a poor prognosis but in otherwise good health.

Approach: Newly diagnosed and previously untreated patients with oat cell and squamous cell carcinomas of the lung will be randomized with due regard for age, sex, and known variables for survival in the specific tumor. BCG will be administered by a schedule to be arranged with the Project Officer. All clinical studies necessary to establish the diagnosis and the extent of the cancer, as well as periodic documentation of the patients' level of health, tumor status, reaction to therapy, deviations from protocol, time to relapse, and survival time will be made. Certain specified immunologic tests will also be conducted.

Progress: During the initial contract period, the contractor has administered 86 aerosolized BCG doses to 20 patients with proven non-resectable lung cancer in a Phase I study. A substantial degree of pulmonary toxicity has been noted in this study, and a protocol for mitigating this toxicity has been developed and tested. The investigators are now ready to undertake a formal, randomized study of this type of treatment in lung cancer patients having a better prognosis.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Because of the possibility that immunotherapy offers a distinct additive to other kinds of therapy, there is an urgent need to evaluate this therapy in patients who are not yet compromised by the extent of their disease.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: May 18, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $101,320

CONTRACT RESEARCH SUMMARY

Title: Preclinical Studies on Tumor Protective Activity of MER

Principal Investigator: David W. Weiss
Name/Address Professor and Chairman, Department of Immunology
Performing Lautenberg Center for General & Tumor Immunology
Organization: Hebrew University - Hadassah Medical School
Jerusalem, Israel
Contract Number: NO1-CB-02208
Starting Date: 06/29/74 Expiration Date: 08/28/75
Goal: Study immunotherapeutic effects of MER and its mechanism of action.

Approach: Evaluation of MER as a therapeutic agent combined with irradiation and/or chemotherapy in prevention of metastatic tumors in mice and guinea pig models, including optimization of dose and schedule of MER. These studies will also be applied to spontaneous mammary tumors and to both mice and guinea pigs subjected to chemical oncogenic agents, early in the induction of tumors and after palpable tumors have arisen. The mode of action of MER will be assayed with a number of different in vitro techniques for cell-mediated and humoral immunity, some of which will utilize tumor cells and specific targets. In addition, the possibility that MER, BCG organisms, certain other subcellular components of BCG and a large variety of taxonomically unrelated bacterial pathogens may share common antigens with a number of different neoplastic cell types is being investigated.

Progress: MER with either irradiation or chemotherapy represents the form of treatment most likely to be effective against solid tumors in the animal models used. Preliminary models for study of the effects of MER alone on pulmonary metastases in MTV mammary carcinomas had been initiated and early evidence that MER plus specific immunization with tumor cells and X-ray therapy could be a valuable form of therapy is being accumulated. Studies in immunoprophylaxis against mice mammary tumors have helped define parameters of MER administration which would not induce enhancement. MER has been demonstrated to be effective as an immunoprophylactic agent in Line 10 hepatoma of Strain 2 guinea pigs; therapy experiments have been initiated. A number of studies directed towards evaluation of the mode of action of MER are in progress and new techniques for these studies are being developed.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Development of preclinical models for immunotherapy and evaluation of mechanism of action in such models is the basis for rational approaches to human immunotherapy.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: September 2, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: 0 (FY74, $90,478 for 14 months)

CONTRACT RESEARCH SUMMARY

Title: Anti-tumor Activity of RNA Extract and Transfer Factor

Principal Investigator: Sheldon Dray, M.D., Ph.D.
Name/Address Professor & Head, Dept. of Microbiology
Performing University of Illinois School of Basic
Organization: Medical Sciences
Chicago, Illinois 60680
Contract Number: N01-CP-23205
Starting Date: 08/01/74 Expiration Date: 08/31/75
Goal: Evaluate immune RNA extracts of lymphocytes for capacity to exert an immunotherapeutic action.

Approach: RNA rich extracts from lymphoid tissues of strain 2 guinea pigs immune to Line 10 hepatoma and from xenogeneic sources are evaluated *in vivo* for therapeutic efficacy.

Progress: The investigators report that established five-day old tumors will regress when treated intralesionally with a mixture of normal peritoneal exudate cells, anti-tumor immune RNA, and 1 ml. soluble tumor-specific antigen. The investigators also have early evidence that tumor nodules remote from the injection site may also regress.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,3)
Immune RNA may be a relatively nontoxic and possibly highly specific approach to immunotherapy of human cancer.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $84,081

CONTRACT RESEARCH SUMMARY

Title: The Immunological Stimulant Properties of Amphotericin B

Principal Investigator: J. Russell Little, M.D.
Name/Address Performing Organization: Jewish Hospital of St. Louis
218 South Kingshighway
St. Louis, Missouri 63110

Contract Number: N01-CB-43872
Starting Date: 03/01/75 Expiration Date: 02/28/76
Goal: To evaluate immunologic responses in animal models receiving Amphotericin B (AmB).

Approach: B and T cell responses will be evaluated in animals receiving AmB following immunizations with soluble hapten protein conjugates. Other aspects of the humoral and cellular immune response will also be evaluated in relation to SRBC administration. The rejection time of histo-incompatible skin grafts and GVH will also be evaluated in reaction to AmB. Macrophage responses and AmB effects on the immunosuppressed animal will also be evaluated.

Progress: The investigators have demonstrated an immuno-stimulant effect in BALB-C mice for humoral immune responses; macrophage activation has been shown by _in-vitro_ and _in vivo_ studies; a toxicity of Amphotericin B for murine thymocytes has been demonstrated; and an increase in the serum level of colony-stimulating factor after administration of Amphotericin B has been found.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
The possibility of using simple chemicals for the stimulation of the immune response must be thoroughly investigated.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $69,465

CONTRACT RESEARCH SUMMARY

Title: Multi-Test Device with Standardized Antigens to Assay Delayed Hyper-Sensitivity via the Skin Test

Principal Investigator: Gary L. Hein
Name/Address President
Performing Lincoln Laboratories, Inc.
Organization: Decatur, Illinois 62525

Contract Number: N01-CB-43954
Starting Date: 06/30/74 Expiration Date: 07/29/75
Goal: To develop a multi-test device to be used in studying skin test responsiveness in patients receiving immunotherapy.

Approach: Standardized viral, bacterial, and fungal antigens will be developed in concentrated form which will permit their use on a multi-puncture skin test device. The skin testing device will be constructed so that multiple antigens may be applied simultaneously in a highly reproducible manner.

Progress: Three antigens have been developed in animal models and are nearly ready for human testing. Food and Drug Administration guidelines are being fulfilled. The multitest device has been developed and will be in production before the end of the first contract year.

Significance for Cancer Research (NCP Objective 6 Approach 1)
The need for standardization of in-vivo testing of patients is a fundamental problem in clinical immunotherapy.

Project Officer: Dorothy Windhorst, M.D. June 13, 1974 and
Program: Immunotherapy Site Visit Date: July 9-10, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: 0 (FY74, $140,118 for 13 months)

CONTRACT RESEARCH SUMMARY

Title: Conduct Bone Marrow Transplantation Studies

Principal Investigator: Name/Address Performing Organization:	Dr. Clement Darrow Litton Bionetics, Inc. 5510 Nicholson Lane Kensington, Maryland 20795

Contract Number: N01-CB-02103
Starting Date: March 9, 1970 Expiration Date: April 8, 1975

Goal: To establish a preclinical model for bone marrow transplantation in rhesus monkeys and to study and modify the graft versus host reaction (GVHR). A subsidiary goal is to study monkey transplantation antigens.

Approach: Rhesus monkeys are lethally radiated and then given histoincompatible bone marrow. Their subsequent course is followed clinically, hematologically, bacteriologically, virologically, chemically, and immunologically. Modification of GVHR is attempted with antilymphocyte serum (ALS) prepared by various methods and given to the recipient pre- and/or post-grafting or used for in vitro incubation of the marrow prior to infusion.

Progress: Multiple variables of a bone marrow transplantation regimen had previously been investigated and studies of allografts have now been completed. These show that no dose of X-ray will permit establishment of a mismatched unrelated bone marrow graft in the dose range $0.5 - 13.9 \times 10^8$/kg without unacceptable extramedullary toxicity. Alternatives for immunosuppression would include X-ray at a lower dose rate, radiation of different energy spectrum, reduction of X-ray dose and addition of other nonspecific immunosuppression, or development of specific measures of immunosuppression. An in vitro assay of hemopoietic stem cell toxicity in antirhesus ALS has been developed; attempts to correlate stem cell toxicity in ALS and removal of this toxicity by absorption with the effect of ALS upon bone marrow autografts in vivo are in progress. A possible homozygote at a putative major locus determining stimulation in the mixed lymphocyte reaction has been identified in one of the monkey families in the breeding colony; further studies may permit analysis of the genetics of this locus. First attempts at identification of the products of a possible "I region" linked to the major histocompatibility locus have been successful using methods worked out in mouse systems. Analysis of the genetics of this system is in progress.

Significance for Cancer Research (NCP Objective 6, Approach 1)
Bone marrow transplantation may allow more intensive cancer chemo- or radiotherapy than is presently possible. Transplantation of bone marrow may be important in cancer immunotherapy.
Project Officer: G. Nocholas Rogentine, Jr., M.D., R. G. Graw, Jr., M.D.
J. R. Neefe, Jr., M.D.
Program: Immunology, Support Site Visit Date: March 1, 1974
Technical Review Group: Ad Hoc Rumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY 75 Funds: $44,000 (estimate)

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. James McCoy

Name/Address
Performing
Organization: Litton Bionetics, Inc.
5510 Nicholson Lane
Kensington, Maryland 20795

Contract Number: N01-CB-23227

Starting Date: March 1, 1972 Expiration Date: August 31, 1975

Goal: To study the immune responses of cancer patients to tumor associated antigens.

Approach: A series of immunological tests have been set up: lymphocyte stimulation by mitogens, recall antigens and tumor cells and extracts; rosette assays for enumeration of T and B cells; leukocyte migration inhibition assays with recall antigens and tumor extracts; delayed hypersensitivity skin tests with tumor extracts; and lymphocyte cytotoxicity assays against tumor cells.

Progress: Emphasis has continued on standardizing each of the assays and in minimizing day-to-day variability. This has been helped by cryopreservation of lymphocytes for the rosette and lymphocyte stimulation assays. The assay for T lymphocytes has shown a decreased number of T cells in the blood of lung carcinoma and Ewing's sarcoma patients and a reduction in percentage of T cells in the blood of patients with breast carcinoma and melanoma. In the leukocyte migration inhibition assay, specific inhibition of migration in a large percentage of cancer patients tested has been shown. This assay can detect cell-mediated immunity against tumor antigens. Cytotoxicity assays, have shown that many normal individuals have as much or even more reactivity than cancer patients. The general immunological competence of cancer patients was monitored in the lymphocyte stimulation assay with general mitogens and mixed leukocyte cultures (MLC). Preliminary studies indicate a suppression of responses to MLC and to certain mitogens in some carcinoma patients. Also, it was shown that allogeneic KCl tumor extracts produced stimulation not only of cancer patients but also of normal individuals, presumably due to the presence of histocompatibility antigens. However, stimulation of lymphocytes from breast, colon and lung carcinoma patients by autologous tumor cells or extracts of these cells has been obtained in approximately 50% of the patients tested, suggesting cell-mediated immune reactivity by the tumor bearing host against tumor antigens. Membrane and KCl extracts have been prepared for use in delayed hypersensitivity skin tests and for migration inhibition and lymphocyte stimulation assays.

Significance for Cancer Research (NCP Objective 5, Approach 4)
This contract provides a comparison between assays of cell-mediated immunity in tumor patients and information on the usefulness of these assays in immunodiagnosis and in following the clinical course of patients.

Project Officer: Ronald B. Herberman, M.D.

Program: Immunology, Support Site Visit Date: June 4, 1974

Technical Review Group: Ad Hoc Tumor Immunology Review Group

Relevance Review Group: DCBD Contract Program Review Committee

FY 75 Funds: $494, 465

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Role of Circulating Tumor Antigens

Principal Investigator: Charles F. McKhann, M.D.
Name/Address Department of Surgery
Performing University of Minnesota Medical School
Organization: Minneapolis, Minnesota 55455

Contract Number: N01-CB-43946
Starting Date: 05/01/74 Expiration Date: 04/30/75
Goal: To study the effects of circulating tumor antigens in relation to tumor size and response to immunotherapy.

Approach: Antigen and antibody complexes in the circulation of normal and tumor-bearing mice will be evaluated in relation to tumor size and in vitro measurements of immune responsiveness. Effects of irradiation in this system will also be studied. Tumor antigen, antibody to tumor antigen, and antigen-antibody complexes will be studied for stimulation and blocking of stimulation of lymphocyte dependent antibody and cellular cytotoxicity.

Progress: Utilizing a mouse system with 2 methylcholanthrene induced tumors the investigator has shown that lymph node cells from tumor-bearing mice will react in vitro to the specific tumor if the tumor load is small. However, lymph node cells from mice with tumors greater than 1 cm. in diameter will not react. Similar results were found using soluble tumor antigens rather than whole tumor cells. Sera from animals bearing large tumors have been found to stimulate lymph node cells from immune mice, suggesting that such sera contains circulating antigens. Tumor immune lymph node cells could be induced to become non-reactive to tumors in vitro by prior exposure to a soluble extract of the same antigen. Preliminary results suggest that macrophages may be playing a role in the specifically non-reactive lymphoid cell populations. Longitudinal studies suggest that systemic immunity is present and vigorous until approximately 15 days after inoculation of tumors in experimental animals. The subsequent dramatic suppression of cellular immunity appears to coincide with the time when tumor antigens can be detected in the circulation.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,3,4)
Kinetics of circulating tumor antigens may have a profound effect on proper design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $85,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy through Inhibition of Antibody Production

Principal Investigator: Charles F. McKharn, M.D.
Name/Address Department of Surgery
Performing University of Minnesota Medical School
Organization: Minneapolis, Minnesota 55455

Contract Number: N01-CP-92061
Starting Date: 06/14/74 Expiration Date: 06/13/75
Goal: Evaluate the role of immunity in the success or failure of tumors and determine how best to manipulate the immune response to the benefit of the host.

Approach: Manipulate the immune response of tumor-bearing animals by developing antibodies against plasma cells and B cells. Evaluate any therapeutic effects of this manipulation in tumor-bearing animals.

Progress: Antibody directed specifically against plasma cells was tested extensively for inhibition of tumor growth. Anti-plasma cell serum (APS) worked well in some tumor systems but not in others. Even where it was effective, a modest increase in the size of the tumor challenge dose overcame the APS inhibition. In contrast, the same cytotoxic anti-sera have been useful when used for in vitro manipulations of anti-tumor immune responses.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,3,4)
Knowledge of how circulating antigens may influence immunotherapy is important for the design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $60,000

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in Tumor Immunology

Principal Investigator:	Jon R. Schmidtke, Ph.D.
Name/Address	Department of Surgery
Performing	University of Minnesota Medical School
Organization:	Minneapolis, Minnesota 55455

Contract Number: N01-CB-43948
Starting Date: 06/01/74 Expiration Date: 05/31/75
Goal: Develop assays and models for evaluation of macrophages in immunotherapy.

Approach: Syngeneic tumors in mice will be utilized to study the role of macrophage function in both the afferent and efferent phases of the immune response. Studies will include evaluation of phagocytosis, catabolism of tumor antigens, the capacity of macrophages to transfer immunogenicity, tumoricidal capacity of macrophages and study of MIF effects. Human macrophages will be evaluated with a number of _in vitro_ assays.

Progress: In the first eight months of the contract, the investigator has developed rabbit and goat anti-mouse gamma globulins and mouse anti-C_3H Balb/c and C57BL/6 anti-sera. A micro-method for the measurement of antibody to human serum albumin has also been developed, as has an indirect assay for the measurement of MIF in mice immunized to _Listeria monocytogenes_. Three syngeneic tumors have been established in mice, and preliminary experiments have been completed on the ability of macrophages from normal and tumor-bearing mice to take up, catabolize and handle two protein antigens. The ability of macrophages to transfer immunogenicity to these protein antigens is now being studied, and the influence of serum from tumor-bearing animals on this macrophage function is being evaluated.

Significance for Cancer Research (NCP Objective2,4,6Approach1,2,4)
Evaluation of macrophage activity is important to the timing of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $70,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Cancer in Man

Principal Investigator: Richard L. Simmons, M.D.
Name/Address Department of Surgery
Performing University of Minnesota Medical School
Organization: Minneapolis, Minnesota 55455

Contract Number: N01-CB-23885
Starting Date: 02/01/75 Expiration Date: 01/31/76
Goal: To test the usefulness of immunization with autochthonous, neuraminidase treated cells in therapy of patients with widely disseminated cancer not amenable to other techniques of treatment

Approach: This will be done by 1) determining the immunologic competence of the patient; 2) determining the immunologic reactivity of the patient to his own and similar tumors; 3) instituting the trial of therapy with neuraminidase treated tumor cells derived from the patient's own cancer; 4) re-determining the patient's immunologic competence at intervals during the course of treatment; 6) utilizing the criteria of objective remission used by cooperative chemotherapy groups in following the tumors in these patients; and 7) discontinuing therapy whenever lack of response is demonstrated. Patients will be limited to those with melanoma and sarcoma who have been demonstrated to have residual tumor that is not amenable to further treatment with either chemotherapy or surgery. In addition, these patients will have been demonstrated to have good immunologic responses.

Progress: In the two years since the investigator has initiated human studies, he has evaluated approximately 86 melanoma patients and 30 sarcoma patients. Early results suggest that the use of neuraminidase treated tumor cells plus BCG may be valuable in Stage II melanoma but not in Stage III. There are also preliminary suggestions that this may be a worthwhile procedure in sarcoma patients with disseminated disease. Approximately two more years of patient entry and follow-up will be required to evaluate these preliminary findings.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Modified autochthonous tumor cells have been shown to be promising immunotherapeutic agents in animals. This contract attempts to apply the animal work to human cancer patients.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: December 5-6, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $102,449

CONTRACT RESEARCH SUMMARY

Title: Graft Versus Tumor: A Model for Treatment of Carcinoma, Leukemia and Lymphoma

Principal Investigator: Mortimer M. Bortin, M.D.
Name/Address Performing Organization: Winter Research Laboratory
Mount Sinai Medical Center
Milwaukee, Wisconsin 53233

Contract Number: N01-CB-33853
Starting Date: 08/25/74 Expiration Date: 08/24/75
Goal: To evaluate adoptive immunotherapy using histo-incompatible immunocompetent cells in the treatment of spontaneous leukemia-lymphoma in AKR mice.

Approach: Using spontaneous tumors in AKR mice: 1) combinations of chemotherapy and chemoradiotherapy will be evaluated for treatment of leukemia and for inducing immunosuppression; 2) H-2 incompatible immunocompetent cells will be administered to the immunosuppressed animals and graft versus host reaction will be monitored; 3) chemotherapy and chemoradiotherapy plus antiserum against the H-2 incompatible cells plus immune H-2 compatible bone marrow will be administered for purposes of rescue from the graft versus host reaction.

Progress: The investigator has established a colony of approximately 1500 AKR mice bearing spontaneous leukemia-lymphoma (SLL) for: treatment with one of 32 regimens employing chemotherapy or chemoradiotherapy; chemotherapy or chemoradiotherapy + transplant of foreign bone marrow cells from healthy mice; and untreated controls. Important findings include the observation that, while single high doses of chemoradiotherapy are associated with high mortality rates, a low initial dose of chemoradiotherapy followed by large doses of chemoradiotherapy are tolerated well, and occasionally, has resulted in marked tumor load reduction. Experiments are underway to administer H-2 incompatible immunocompetent cells to immunosuppressed leukemia-lymphoma mice with subsequent monitoring of graft versus host reactions and their effects on tumor growth. Early results have been encouraging in 2 groups. Evaluation of chemotherapy or chemoradiotherapy and antiserum against H-2 incompatible cells + administration of immune H-2 compatible bone marrow for purposes of rescue from the graft versus host reaction is being undertaken.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Development of an animal model with immunocompetent cells with "rescue" from graft versus host disease could be highly effective in human immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $75,000

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigator: James F. Holland M.D. and J. George Bekesi Ph.D.
Name/Address Mt. Sinai School of Medicine
Performing 5th Avenue and 100th Street
Organization: New York, New York 10029

Contract Number: N01-CB-43879
Starting Date: 06/30/74 Expiration Date: 12/20/75
Goal: Evaluate Neuraminidase-treated allogeneic cells and MER in human acute myelocytic leukemia (AML).

Approach: Neuraminidase (VCN) treated allogeneic cells and MER will be evaluated in an appropriately designed, randomized study of AML patients after remission induction with cytosine arabinoside and Daunorubicin. Patients will be studied with both *in vivo* and *in vitro* immunologic tests.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Suggestive early leads in immunotherapy of AML require that vigorous application of other possible modalities to this disease be undertaken.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: April 5, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: 0 (FY74, $454,182 for 18 months)

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor Therapy of Sarcoma

Principal Investigator: Albert F. LoBuglio, M.D.
Name/Address Ohio State University Research Foundation
Performing 410 West 10th Avenue
Organization: Columbus, Ohio 43210

Contract Number: N01-CB-43878
Starting Date: 04/01/74 Expiration Date: 03/31/75
Goal: Evaluate transfer factor as a therapeutic agent in human sarcomas.

Approach: Transfer factor will be derived by a chromatographic isolation technique from the leukocytes of relatives of tumor patients who have been shown to have tumor immunity with in vitro assays. Several different types of sarcomas will be studied in appropriately designed protocols.

Progress: The investigator has undertaken clinical protocols to treat bone and soft tissue sarcomas with transfer factor immunotherapy to reduce the incidence of tumor recurrence in patients undergoing curative resection. He is on schedule in producing a pool of transfer factor from the patients' immunocompetent relatives. The therapy strategy is to administer large amounts of transfer factor in a short time to patients in the postoperative period (1-3 months post-op) in order to accentuate tumor immunity when tumor cell numbers should be at a minimum. Clinical and laboratory evaluation on specific tumor immunity and general immune evaluation is proceeding satisfactorily. The investigator is meeting his patient accrual rate of 10 patients with osteosarcoma and 20 with "other" sarcomas per year.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Transfer factor may be a relatively nontoxic and possibly highly specific approach to immunotherapy of cancer.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $145,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Development of Animal Models for Evaluation of Therapy with Neuraminidase Treated Tumor Cells

Principal Investigator: G. Mark Kollmorgen, Ph.D.
Name/Address Performing Organization: Oklahoma Medical Research Foundation
825 Northeast 13th Street
Oklahoma City, Oklahoma 73104

Contract Number: N01-CB-43864
Starting Date: 11 /1/74 Expiration Date: 10/31/75

Goal: To explore the parameters of therapy with neuraminidase treated autologous or isogeneic tumor cells.

Approach: Using known successful animal models, parameters of immunotherapy with tumor cells such as cell number, route and schedule of administration, methods and duration of cell storage, and use of tissue culture cells will be studied. In addition, potential new model systems for evaluation of this form of immunotherapy will be explored. Such systems will be required to have high applicability to human cancer; that is, therapy will be evaluated in the presence of established tumors and the tumors will be of relatively recent origin.

Progress: The contractor has made significant progress in the workscope relating to the combination of chemotherapeutic agents and immunotherapeutic agents in one tumor model. The information he has gained from this model will now be applied to other tumor systems.

Significance for Cancer Research (NCP Objective 6 Approach 1, 4)
Animal models for immunotherapy will not only contribute to the understanding of immunotherapy but also to the problems of diagnosis of the quantity of malignant disease and the evaluation of normal body mechanisms of defense against malignant disease.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: May 21, 1973
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $135,451

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in the Immune Response to Tumors (Immunotherapy)

Principal Investigator: James L. Krahenbuhl, Ph.D.

Name/Address Performing Organization:
Palo Alto Medical Research Foundation
860 Bryant Street
Palo Alto, California 94301

Contract Number: N01-CB-43873

Starting Date: 05/01/74 Expiration Date: 04/30/75

Goal: To study the role of macrophages in the immune response to tumors, particularly in relation to immunotherapy, both in animals and in man.

Approach: Guinea pig and mouse peritoneal macrophages and human peripheral blood monocytes and monocyte derived macrophages will be studied for their capacity to adversely affect a number of syngeneic and allogeneic tumor target cells. Kinetics of the response of activated macrophages will be examined and the macrophage activation related to acute and chronic stages of infection will be evaluated. Cell mediated immune responses of animals and patients to Toxoplasma antigens, streptokinase-streptodornase, and PPD will be evaluated by skin tests and by *in vitro* techniques.

Progress: Cytostatic effects of peritoneal macrophages and peritoneal lymphocytes have been compared in animals infected with intracellular protozoans. Macrophages were found to be nonspecifically cytostatic for a variety of tumor cells. Infection with *Listeria* or treatment with C. parvum was found to induce a transient state of macrophage activation when compared with infection with Toxoplasma. The cytostasis appears to result from direct contact between the activated macrophage and target cells. Several methods designed to evaluate viability of target cells have shown that activated macrophages appear to be highly cytostatic but not cytotoxic.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Understanding mechanisms of macrophage activity has high relevance to the timing of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.

Program: Immunotherapy Site Visit Date: None

Technical Review Group: Committee on Cancer Immunotherapy

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: Estimated $126,000

CONTRACT RESEARCH SUMMARY

Title: Cancer Immunotherapy: Animal Models for Treatment of Minimal Residual Systemic Tumor
Principal Investigator: Gerald L. Bartlett, M.D.,Ph.D.
Name/Address M. S. Hershey Medical Center
Performing Pennsylvania State University
Organization: Hershey, Pennsylvania 17033

Contract Number: N01-CB-33891
Starting Date: 11/01/74 Expiration Date: 10/31/75
Goal: To develop animal models for the assessment of the usefulness and parameters of administration of various types of immune stimulants as therapy for cancer.

Approach: Animals models with a direct applicability to human cancer will be studied for the effectiveness of various immunostimulants on their cancers. The immunotherapy will be given after tumor load has been reduced by surgery, radiation, or chemotherapy, and the tumor load will be quantifiable. Experimental variables will include strain of organism, dose, schedule, and route of administration.

Progress: During the past year the investigators have searched for an experimental tumor system responsive to immunotherapy. Three murine tumor models were evaluated: the B16 melanoma of C57 BL/6J mice, the LSTRA lymphoma of Balb/c mice, and the CaD_2 transplanted mammary carcinoma of DBA/2J mice. Systemic administration of BCG was not effective treatment for mice with the B16 melanoma or mice with the LSTRA lymphoma. Intralesional injection of Bordatella pertussis into the mammary carcinoma retarded tumor growth. The investigators have gained valuable experience in the problems involved in identifying an experimental tumor that responds to systemic immune therapy. During the second contract year the investigators will concentrate on three animal tumors known to be responsive to immune therapy: the 13762 transplantable mammary carcinoma of Fisher 344 rats; the line 10 hepatoma of strain 2 guinea pigs; and the LSTRA lymphoma of Balb/c mice.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Evaluation of parameters of promising forms of immunotherapy in animals is necessary for optimum utilization in humans.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: May 30, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $164,380

CONTRACT RESEARCH SUMMARY

Title: Hapten Treatment of Cancer

Principal Investigator: Kazuhiko Arai, M.D.
Name/Address The Graduate Hospital
Performing University of Pennsylvania School of Medicine
Organization: Philadelphia, Pennsylvania 19146

Contract Number: N01-CB-43967
Starting Date: 05/16/74 Expiration Date: 05/15/75
Goal: To evaluate L-phenylalanine plus antiserum in a syngeneic rat system.

Approach: Protocols utilizing rats which have previously indicated that intratumoral injection of L-phenylalanine mustard plus I.V. injection of antiserum against tumor modified with L-phenylalanine mustard has a therapeutic effect will be used to study syngeneic rat tumors *in vivo*.

Progress: During the initial contract period the investigator has established two syngeneic tumors in two Lewis rat strains and has evaluated 1) methods of tumor preparation for inoculation; 2) the genetic differences between the two rat strains; 3) growth rates for the tumors and survival of tumor-bearing rats; 4) methods of preparing tumor extract for conjugation with L-phenylalanine mustard (PHM); and 5) the effects of preparation techniques on the antigenicity of the conjugates. These first techniques for preparation of the conjugate yielded a weakly antigenic material and, of four rats who received therapy with this material, tumor growth rates were significantly less than in controls. A new tumor conjugate of greater antigenicity has also been developed. It is expected that in the next contract year the present study will be completed, the studies will be repeated using xenogeneic antisera, and the effects of the antisera on uninjected nodules will be evaluated.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,4)
This work will evaluate a promising animal model in a system more applicable to human tumors.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $56,000

CONTRACT RESEARCH SUMMARY

Title: Neuraminidase Effectiveness in Tumor Immunotherapy and Mechanism of its Effect
Principal Investigator: Gabriel J. Gasic, M.D.
Name/Address Department of Pathology
Performing University of Pennsylvania School of Medicine
Organization: Philadelphia, Pennsylvania 19104

Contract Number: N01-CB-33860
Starting Date: 08/25/74 Expiration Date: 08/24/75

Goal: To evaluate neuraminidase treated tumor cells in immunotherapy by establishing several animal tumor models and then examining the variables of dose, route of injection, etc., in these models. To examine the capacity of neuraminidase treatment in therapy of postsurgical metastatic cancer.

Approach: 1) Test the effectiveness of neuraminidase-treated tumor cells as immunotherapy in two spontaenous and three methylcholanthrene induced mouse tumors. 2) Using model systems in which the neuraminidase treatment is effective, the following parameters will be evaluated: dose of treated tumor cells, numbers of successive subcutaneous injections, route of treatment, and time of administration after tumor grafting. 3) Evaluation of the effectiveness of neuraminidase-treated tumor cells in immunotherapy of metastatic tumor using metastatic tumor cells. 4) Examination of the effectiveness of neuraminidase modified cells in relation to the natural immunogenicity of certain target tumors.

Progress: Successful application of cholera neuraminidase (VCN) in cancer therapy has been limited to a few tumor systems. To investigate whether other tumors may also be susceptible to this therapy, chemical tumors were induced in Balb/c (12A9 urinary bladder carcinoma), B10D2 new (MC2 fibrosarcoma), C3H/He (MC43 fibrosarcoma), and in C57BL/6 (MC1 fibrosarcoma) mice. Two spontaneous tumors (Mammary #4 and #5) originated in C3H/He female mice. In 8 experiments, mice were treated with 6 injections s.c. every other day of 10^6 VCN- and mitomycin-treated tumor cells. In one experiment B10D2 new and B10D2 old mice received only 3 injections of the immunogen. Controls were injected with medium M199 or with tumor cells incubated with heat-inactivated VCN and mitomycin C. Results indicate that injection of VCN-treated tumor cells: 1) achieved no beneficial therapeutic effect in mammary tumors #4 and #5 and in 12A9 tumor; 2) induced no tumor regression but reduced tumor growth &/or prolonged survival. This was observed in MC43 fibrosarcoma and MC2 fibrosarcoma; and 3) produced some total tumor regression or contributed to tumor growth slowdown &/or prolonged survival in MC1 fibrosarcoma, a highly immunogenic tumor.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Manipulation of autologous tumor with neuraminidase in animals has direct relevance to tumor immunotherapy in man.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $32,933

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Accessible Neoplasms and Analysis of Immune Status
In vivo
Principal Investigator: Edmund Klein, M.D.
Name/Address Research Foundation of the State University of New York
Performing Roswell Park Memorial Institute
Organization: Buffalo, New York 14203

Contract Number: N01-CP-33373
Starting Date: 03/21/75 Expiration Date: 02/20/76
Goal: Exploratory studies in the utilization of topical immunotherapy and correlation with in vivo measurement of immune status.

Approach: Patients with various neoplasms originating or metastasizing to the skin will be entered into specific clinical protocols for application of topical immunotherapeutic agents. Some patients will receive BCG by scarification. Multiple antigens will be used for skin testing on all patients and the skin reactivity will be correlated with clinical status and immunotherapy administration.

Progress: The contractor has developed clinical protocols and entered patients into these protocols as follows: 1) Protocol #438-BCG Effects on Skin Test Responses and Incidence of Recurrence in Surgical Remission of Malignant Melanoma. Thirty five patients have been entered in this study distributed as follows: 13 controls, 12 BCG intradermally, and 10 BCG orally. All patients receiving BCG had increased skin test responses. Some patients receiving oral BCG have shown temporary depression of skin test responses (PPD & unrelated antigens). 2) Protocol #69-273-Double Blind Comparison of Topical 5-FU, PPD, and Combination of 5-FU/PPD on Cutaneous Neoplasms. Thirteen patients have been enrolled in this study since its August, 1974, initiation, and more time and patients will be required to make statements about results under this protocol. 3) Protocol #442A and 442B-Studies on Breast Cancer. This protocol has not been initiated because of lack of patients and will not be supported by this contract. The contractor has also initiated a program to provide for statistical evaluation of skin test responses to PPD and unrelated antigens in protocol patients.

Significance for Cancer Research (NCP Objective 2,5,6 Approach 1,3,4)
Detailed information regarding results of skin testing in relation to clinical status and therapy provide vital feedback for therapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D. and Charles Boone, M.D.
Program: Immunotherapy Site Visit Date: October 31, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $74,147

CONTRACT RESEARCH SUMMARY

Title: Macrophage Activation in Tumor Immunity and Immunotherapy of Rat Mammary Tumor
Principal Investigator: W. Hallowell Churchill, Jr., M.D.
Name/Address Performing Organization: Robert B. Brigham Hospital
125 Parker Hill Avenue
Boston, Massachusetts 02120

Contract Number: N01-CB-33896
Starting Date: 11/01/74 Expiration Date: 10/31/75
Goal: To study the mechanism of tumor immunogenicity in order to design a more effective and rational program of tumor immunotherapy in man.

Approach: The contractor will evaluate the ability of macrophages to kill tumor cells after *in vitro* activation by lymphocyte mediators, and determine whether macrophages activated by adjuvants *in vivo* show differences in tumor cell killing activity *in vitro*. In addition, he will extend these studies to human monocytes.

Progress: During the past contract period, the investigators have established new systems for the study of macrophage cytotoxicity for tumor cells that will facilitate dissection of the mechanisms of tumor cytotoxicity and how such mechanisms might be stimulated for immunotherapeutic purposes. In addition, substantial progress has been made in establishing a rat mammary carcinoma system for experiments in immunotherapy.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Immunotherapeutic manipulations in animals are needed to evaluate multiple parameters of immunotherapy that cannot satisfactorily be tested in humans.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $171,916

CONTRACT RESEARCH SUMMARY

Title: Subcellular Fractions and Immunotherapy: effect of transfer factor on virus-induced tumors in marmoset monkeys.

Principal Investigator: Peter Baram, Ph.D.
Name/Address Performing Organization: Div. of Microbiology & Immunology
Rush-Presbyterian-St. Luke's Medical Center
Chicago, Illinois 60612

Contract Number: N01-CB-43951
Starting Date: 06/28/74 Expiration Date: 06/27/75
Goal: Utilize a subhuman primate system to evaluate a promising mode of immunotherapy.

Approach: Two different virus-induced tumors of marmoset monkeys will be utilized to evaluate therapeutic effect of transfer factor, its specificity, and the optimum dose and schedule of administration. Transfer factor will be compared with immune RNA in one of these studies.

Progress: The investigator has early evidence suggesting that he has achieved lymphocyte transformation with marmoset lymphocytes; acquired reproducibility in the marmoset MIF assays; and achieved transfer of delayed hypersensitivity to KLH as measured by the MIF assay in two of four rhesus monkeys using KLH reactive dialyzable transfer factor (TF_d).

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Subcellular fractions such as transfer factor and immune RNA may be relatively nontoxic and possibly highly specific approaches to cancer immunotherapy. Lack of suitable models in smaller animals requires that primate systems be studied.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: April 29, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: Estimated $97,000

CONTRACT RESEARCH SUMMARY

Title: Role of Circulating Tumor Antigens (Immunotherapy)

Principal Investigator:	Joseph D. Feldman, M.D.
Name/Address Performing Organization:	Scripps Clinic and Research Foundation 476 Prospect Street La Jolla, California 92037

Contract Number: N01-CB-43874

Starting Date: 03/01/75 Expiration Date: 02/28/76

Goal: To evalute the effects that circulating tumor antigens may have on immunotherapeutic maneuvers.

Approach: Several different antigens associated with a viral tumor system will be isolated. The metabolism of these antigens and the circulation in hosts with regressing and progressing tumors will be measured. The immune response to each category of antigen will be analyzed and the antigen will be utilized to evaluate their capacities to accentuate destruction of the tumor.

Progress: The progress made on this contract in the first contract period of performance is of a very high caliber. In particular, the methods for purifying membrane antigens and preparing anti-sera specific for these antigens have been developed to an advanced degree.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,3,4)
Knowledge of how circulating antigens may influence immunotherapy is important for the design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.

Program: Immunotherapy Site Visit Date: None

Technical Review Group: Committee on Cancer Immunotherapy

Relevance Review Group: Tumor Immunology Coordinating Committee

FY75 Funds: $94,144

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Human Cancer

Principal Investigator: Jordan U. Gutterman, M.D.
Name/Address M. D. Anderson Hospital & Tumor Institute
Performing University of Texas System Cancer Center
Organization: Houston, Texas 77025

Contract Number: NO1-CB-33888
Starting Date: 10/25/74 Expiration Date: 10/24/75
Goal: To evaluate BCG as a form of immunotherapy in several types of local and disseminated human cancer.

Approach: Patients with wide-spread involvement of a given cancer in relatively good condition but with a uniformly poor prognosis will be randomized with due regard for age, sex, general condition, and other variables determined by the particular tumor. Statistical considerations to insure evaluable results will enter into the randomization and the determination of patient numbers to be entered into protocols.

Progress: Three hundred eighty three patients were entered into the various clinical protocols during the previous contract year, and one hundred sixty-one patients previously entered in these protocols were followed under the contract. Substantial information relating to the usefulness of immunotherapy in melanoma and in leukemia has been obtained and utilized in the design of new protocols. Appropriate beginnings have been made in protocols for breast cancer and squamous cell carcinoma.

Significance for Cancer Research (NCP Ojbective 6 Approach 1,4)
Because of the wide-spread casual application of immunotherapy to human patients, it is important to establish well designed ethical clinical protocols.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: March 26, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $375,000

CONTRACT RESEARCH SUMMARY

Title: Study Mycobacteria as Inhibitors of Tumor Cell Growth

Principal Investigator: George B. Mackaness, Ph.D., M.D.
Name/Address Performing Organization: Trudeau Institute Inc.
Immunobiological Research Laboratories
Saranac Lake, New York 12983

Contract Number: N01-CP-23221
Starting Date: 02/01/75 Expiration Date: 01/31/76
Goal: Evaluate various highly characterized strains of BCG for their capacity to suppress tumor growth in guinea pigs and mice; compare C. parvum with BCG in the model systems.

Approach: The biological properties of different strains of BCG grown under different circumstances and preserved in various ways will be examined in terms of immunopotentiating and tumor-suppressive activities.

Progress: The immunotherapeutic and immunopotentiating properties of five strains of fresh-frozen (FF) and freeze-dried (FD) BCG vaccines have been compared by three different assay procedures. C. parvum has been compared with these strains of BCG in one tumor system.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Preclinical evaluation of mechanisms of action and comparative efficacies of promising immunotherapeutic agents is necessary for efficient design of clinical trials.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $172,000

CONTRACT RESEARCH SUMMARY

Title: The Production of an Improved BCG Vaccine with a Minimal Content of Extraneous Material and Improved Immunotherapeutic Potency
Principal Investigator: George B. Mackaness, M.D., Ph.D.
Name/Address Performing Organization: Trudeau Institute, Inc.
Immunobiological Research Laboratories
Saranac Lake, New York 12983

Contract Number: N01-CB-53885
Starting Date: 08/23/74 Expiration Date: 08/22/75
Goal: Develop production methods for an improved BCG which will meet FDA standards for human use.

Approach: BCG is grown in suspension culture in the absence of any animal proteins or other significantly antigenic materials. It is harvested at a defined stage of growth and frozen for distribution for clinical studies.

Progress: The first batches of the fresh frozen BCG suitable for human use under an FDA Investigational New Drug permit are being distributed to clinical investigators.

Significance for Cancer Research (NCP Objective 6 Approach 1)
The superiority of a specific preparation of this agent for immunotherapy has been suggested by preclinical experiments and human trials are awaiting large scale production of this material.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $80,288

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutical Trials with Human Tumors

Principal Investigator:	Dr. Karl Erik Hellström
Name/Address	Department of Pathology
Performing	University of Washington School of Medicine
Organization:	Seattle, Washington 98195

Contract Number: N01-CB-23887
Starting Date: 10/30/74 Expiration Date: 09/30/75
Goal: To evaluate the use of "deblocking" serum as well as immunization with autochthonous tumor cells as possible therapeutic approaches in the treatment of malignant melanoma. BCG may also be used.
Approach: Preliminary studies will be carried out to assure that neither of the immunotherapeutic maneuvers appears to have toxic effects on recipients. After this has been established, a double-blind immunotherapy trial will be established wherein patients will be assessed to determine whether their serum contains blocking antibodies in the colony inhibition assay. Patients with blocking antibodies will be randomized into a test group which will receive deblocking plasma and a control group which will receive normal plasma. Patients whose serum does not contain blocking factor will be randomized into a treatment group that will receive a course of immunotherapy involving active immunization with intradermal inoculations of tumor cells mixed with living BCG, and a control group which will receive no additional therapy. A variety of laboratory investigations will be carried out on these patients.

Progress: As of October, 1974, these studies have accrued 28 patients, eight with Stage II disease and twenty with Stage III disease. There has, as yet, been no striking difference between the experimental and control groups and the code relative to which patients are receiving normal plasma and which are receiving unblocking plasma has not yet been broken.

Significance for Cancer Research (NCP Objective 6 Approach 4)
This contract will provide indepth laboratory analyses of patients' immune responses during immunotherapy and coordination of these responses with clinical course.
Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: October 4, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $169,405

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Mechanism of Action of Immunopotentiators

Principal Investigator: Alan M. Kaplan, Ph.D.
Name/Address Medical College of Virginia
Performing Virginia Commonwealth University
Organization: Richmond, Virginia 23298

Contract Number: N01-CB-43877
Starting Date: 03/15/75 Expiration Date: 03/14/76
Goal: To evaluate the immunologic effects of immunpotentiators in relation to their immunotherapeutic capacity.

Approach: The immunologic action of selected immunopotentiators will be evaluated in model systems for antibody production and cellular immunity. The changes in these responses towards tumors from tumor bearing mice will be measured and compared with the model system. In addition, the effectiveness of the immunpotentiators in the treatment of tumors after using surgery or chemotherapy to remove the bulk of the tumor will be evaluated.

Progress: After screening several synthetic and biologic immunopotentiators, the investigators have selected pyran copolymer, polyacrylic acidmaleic anhydride, and C. parvum for indepth studies of modes of action of immunopotentiators on two tumor systems: Lewis lung carcinoma and the MCA 2182 fibrosarcoma. Pyran has been shown to enhance primary and secondary IgM and IgG response of mice to sheep erythrocytes in a way that requires T lymphocytes. On the other hand, this agent does not enhance cytotoxic antibody production to mastocytoma cells. These and other data are suggesting that complex interrelationships are involved in the action of immunopotentiators. The investigators have made the interesting observation that tumor growth is inhibited and survival time of mice increased in T-cell deprived mice bearing tumors, but that the tumor growth rate returns to normal after inoculation of syngeneic thymus cells. Further, the investigators have early evidence that pyran-activated macrophages administered to mice bearing metastatic Lewis lung tumor may function in a positive therapeutic manner. This and other evidence regarding macrophage infiltration around periphery of tumors suggests that macrophages may be a major factor in the anti-tumor action of certain immunopotentiators, particularly in the case of solid tumors.

Significance for Cancer Research (NCP Objective 4,6 Approach 1,3,4,6)
Understanding the mechanism by which immunpotentiators act is necessary for optimum manipulation of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: February 21, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $99,579

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Development of Therapy with Neuraminidase Treated Tumor Cells
Principal Investigator: Richard K. Gershon, M.D.
Name/Address Performing Organization: Yale University School of Medicine
310 Cedar Street
New Haven, Connecticut 06510

Contract Number: NO1-CB-43865
Starting Date: 11/08/74 Expiration Date: 11/07/75
Goal: A systematic evaluation of various animal tumors for use in testing of therapy with neuraminidase treated tumor cells.

Approach: Tumor models closely resembling human tumors will be established by various well known means. Neuraminidase and other glycosidases will be tested to determine if they have any effect on the capacity of tumor vaccines to effect tumor regression. Dosage and route of administration of such vaccines will be maximized. Nonviable tumor cells and tumor cell fraction vaccines will be evaluated. Combination therapy with vaccine and other modalities will also be pursued.

Progress: Work performed under this contract is contributing significantly to goals within the immunotherapy program relating to understanding mechanisms of action of cell surface modification in immunotherapy. The investigator is one of several who are working with different tumor systems in animal models to evaluate neuraminidase treatment of cells as an immunotherapeutic maneuver.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Preclinical models of immunotherapy are highly important for optimizing human protocols.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: None
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $100,613

CONTRACT RESEARCH SUMMARY

Title: NCI Histocompatibility Typing Center

Principal Investigator: Dr. Frances E. Ward
Name/Address Duke University
Performing Durham, North Carolina 27706
Organization:

Contract Number: (no negotiated contract as yet)
Starting Date: Approx. April 1, 1975 Expiration Date: April 1, 1976

Goal: To study HL-A and other cell surface antigens on normal and neoplastic cells.

Approach: Complement dependent cytotoxicity studies will be performed on human peripheral blood lymphocytes and leukemic cells using antisera and cells provided by the Project Officers. Lymphocyte defined antigens will be investigated by mixed lymphocyte culture tests using DNA synthesis as a measure of detection.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3, Approach 5)
Development of methodologies in the identification of isolated cell surface antigens is a necessary prerequisite to the identification of tumor specific associated antigens.

Project Officer: G. Nicholas Rogentine, Jr., M.D., Dean L. Mann, M.D.
Program: Immunology, Support Site Visit Date: Waived by memo to
Technical Review Group: Ad Hoc Review Group Dr. Berlin 3/31/75
Relevance Review Group: Staff
FY75 Funds: $220,000 (estimate)

CONTRACT RESEARCH SUMMARY

Title: Study of the Immune Responses of Mice and Rats to Tumor Associated Antigens

Principal Investigator: Name/Address Performing Organization:
Dr. David Lavrin
Litton Bionetics, Inc.
5510 Nicholson Lane
Kensington, Maryland 20795

Contract Number: N01-CB-23878
Starting Date: April 28, 1974 Expiration Date: April 27, 1975

Goal: Study of immune responses of mice and rats to tumor associated antigens. Studies on in vivo resistance to tumors and correlation with in vitro cellular and homoral immune responses.

Approach: The kinetics of the cellular and humoral immune responses to tumor-associated antigens of the following tumors will be studied: tumors induced by polyoma virus, SV40, Gross, and other murine leukemia viruses, murine sarcoma virus, and mammary tumor virus; mouse plasma cell tumors; methylcholanthrene-induced tumors.

The specificity of each of the detected antigens in both the humoral and cell-mediated immune studies are being studied by inhibition assays. The nature of the cells involved and the mechanism of cytotoxicity in the cell-mediated cytotoxicity assays are also being studied.

Progress: A major effort has been put into a continuation of the studies of murine sarcoma virus (MSV)-induced cellular and humoral immunity in mice and of Gross tumors in rats. *In vitro* and *in vivo* studies of Freund leukemia antigens have been carried out. In each of these systems, the kinetics of the cytotoxic cell-mediated immune response to the tumor-associated antigens has been determined, both in animals bearing progressively growing tumors and in those with tumor regression. Lymphoid cells from various time points, with known *in vitro* reactivity, were adoptively transferred and the degree of protection against tumor growth measured. A good correlation has been found between the protective effects of the cells and both the tumor status of the donors and the *in vitro* cytotoxic activity of the cells. Natural cell-mediated immunity to type C virus-associated antigens has also been found in these systems. Athymic, nude mice have high levels of natural cytotoxic reactivity.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Understanding of realationship of *in vitro* assays to host resistance to tumors is an essential element in properly using these assays to monitor response to immunotherapy.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology, Support Site Visit Date: February 1974
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY 75 Funds: This contract will terminate April 27, 1975

CONTRACT RESEARCH SUMMARY

Title: Procurement of Melanoma Cell Vaccine and In Vitro Assays for Humoral and Cellular Cytotoxicity

Principal Investigator:	Dr. Edwin Matthews
Name/Address	Litton Bionetics
Performing	5510 Nicholson Lane
Organization:	Kensington, Maryland 20795

Contract Number: NCI-CB-53916-31 (Continuation of Contract N01-CB-02207)
Starting Date: April 28, 1975 (estimate) Expiration Date: April 27, 1976

Goal: To prepare cultured melanoma cells for immunotherapy vaccine and carry out in vitro cytotoxicity tests to measure response of patients to the vaccine.

Approach: 1) Grow human melanoma cells free of contaminants, treat cells with neuraminidase, freeze under sterile conditions, and provide cells as needed for human immunotherapy. 2) Carry out tests for direct cell-mediated cytotoxicity, lymphocyte-dependent antibody and complement-dependent antibody.

Progress: New Contract

Significance for Cancer Research(NCP Objective 6, Approach 1)
The melanoma cell line vaccine will be used in a clinical immunotherapy trial.

Project Officer: William D. Terry, M.D., John R. Wunderlich, M.D.
Program: Immunology, Support Site Visit Date: Waived by memo dated 2/26/75
Technical Review Group: Ad Hoc Review Committee
Relevance Review Group: Staff
FY75 Funds: $270,000 (estimate)

CONTRACT RESEARCH SUMMARY

Title: In Vitro Tests for Tumor Specific Antigens, Antibodies and Immune Cells with Animal and Human Serum Cells

Principal Investigator: Dr. Edwin Matthews
Name/Address
Performing
Organization:
Litton Bionetics, Inc.
5510 Nicholson Lane
Kensington, Maryland 20795

Contract Number: N01-CB-02207
Starting Date: May 28, 1970 Expiration Date: April 27, 1975

Goal: To prepare cultured melanoma cells for immunotherapy vaccine, to study development and function of cell-mediated immune cytotoxicity in humans, and to assay viral infected human cell lines for leukemia associated antigens.

Approach: 1) Grow human melanoma cells free of PPL. Treat with neuraminidase and freeze under sterile conditions. Test for specificity and pyrogenicity. Provide for human immunotherapy. Also, adapt for culture explants of NCI melanoma patients. 2) Study direct cellular cytotoxicity of human lymphocytes. Evaluate lymphocyte dependent antibody with ^{51}Cr release assays. Immunize normal human peripheral blood lymphocytes in vitro. Study antitumor immunity in mice with progressively growing lethal tumors. 3) Murine leukemia viruses are used to infect human nonlymphoid cell lines. Cells are assessed for acute leukemia associated antigen. Cell membranes are solubilized and analyzed by gel electrophoresis.

Progress: The contractor has done well in responding to the Project Officer's needs for increasing amounts of melanoma cell vaccine. In vitro immunologic cytotoxicity studies of melanoma patients have been of high quality and have been kept up to date fairly well. Extensive studies have been carried out on human lymphocytes which are cytotoxic for sensitized target cells and on mouse lymphoid cells cytotoxic for freshly explanted syngeneic tumors.

Significance for Cancer Research (NCP Objective 6, Approach 1)
Melanoma cell line material will be used for a clinical immunotherapy trial. In vitro immunization may boost an inadequate or blocked immune system. Leukemia associated antigen may be useful in finding etiologic agent for human leukemia.

Project Officer: Drs. William D. Terry, Dean L. Mann, and John R. Wunderlich
Program: Immunology, Support Site Visit Date: February 1974
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY75 Funds: Supplement - $20,000

CONTRACT RESEARCH SUMMARY

Title: Immunologic Studies of Murine RNA Oncogenic Viruses and Their Induced Tumors

Principal Investigator: Dr. James McCoy
Name/Address Litton Bionetics
Performing Kensington, Maryland
Organization:

Contract Number: N01-CB-02320
Starting Date: June 29, 1972 Expiration Date: March 28, 1975

Goal: Conduct immunologic studies of tumors induced by RNA oncogenic viruses and the immune responses they engender.

Approach: Newly induced neoplasms in inbred mice and hamsters are used following transformation in vitro and in vivo by MSV pseudotype viruses. Attempts are being made to evaluate the role played by new "cellular" antigens of the membrane or of viral antigens. Selection of "nonproducer" neoplasms is being attempted. Comparison of the in vivo immune responses to such antigens will be made with in vitro colony inhibition, ^{51}Cr release, proline release, and microcytotoxicity assays.

The role of sensitized lymphoid cells and of antibody is being evaluated in the autochthonous host in the induction process of leukemia and of solid tumors using MSV and MSV(MLB).

Progress: Work during this contract period was conducted in close cooperation with the Project Officer and concerned the identification and characterization of TSTA on mineral oil and virus-induced plasmacytomas of mice. A study of the activity of soluble tumor antigens from RNA virus- and DNA virus-induced neoplasms was also undertaken. This part of the work entailed development and standardization of in vitro assays: inhibition of migration of PE cells (an agarose droplet micro method) and lymphocyte stimulation. In addition, further studies of virus "nonproducer" neoplasms and the presence of TSTA of such neoplasms were carried out.

Significance for Cancer Research (NCP Objective 3, Approach 5)
Knowledge gained through a study of the biology and chemistry of specific tumor antigens on the cell surface of neoplastic cells, transformed by RNA and DNA viruses, will hopefully lead to an understanding of the mechanisms of cancer induction and the prevention and control of neoplasms in man.

Project Officer: Dr. Lloyd W. Law
Program: Immunology, Support Site Visit Date: September 1973
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Revelance Review Group: DCBD Contract Program Review Committee
FY75 Funds: $48,949 - additional supplement being negotiated

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation and Preservation of Plasma Cell Tumors in Mice

Principal Investigator: Dr. Kyle Sibinovic
Name/Address Litton Bionetics
Performing Bethesda, Maryland 20014
Organization:

Contract Number: NO1-CB-92142
Starting Date: June 23, 1969 Expiration Date: January 31, 1976

Goal: Induction, transplantation and preservation of plasma cell tumors in mice.

Approach: Contractor preserves and transplants plasmacytomas in a liquid nitrogen bank and provides and ships these tumors on request to the NCI, and other investigators working with mouse plasmacytomas. In addition the contractor induces new plasmacytomas. Series of plasmacytomas producing antigen-binding myeloma proteins for the same hapten are being collected. Myeloma proteins from sources other than BALB/c are also being collected. Contractor assists project officer in the study of the mechanism of priming and tumor specific immunity to plasmacytomas. Contractor provides ascites containing myeloma proteins to NCI.

Progress: In the contract year 169 requests for 337 tumors were received from NIH, (non-NIH) U.S., and foreign investigators. The contractor processed these tumors for distribution. The tumor bank has been expanded to include thymus-derived lymphocytic neoplasms, nonthymus derived lymphocytic neoplasms (eg. B-cell lymphomas induced by Abelson virus), new mastocytomas and reticulum cell sarcomas. The contractor is developing its capability to perform several additional characterizations on tumors in the bank, and it has performed and completed a series of plasmacytoma induction experiments. Viability control experiments have been initiated, and it is hoped that this will lead to improved methods of freezing and recovery of tumors. The contractor has greatly increased its proficiency in quality control evaluation, and the data retrieval system is being improved by the institution of a computer system. The contractor has housed a colony of 5,800 mice and delivered them on request to the Project Officer.

Significance for Cancer Research (NCP Objectives 6, 4, Approaches 1,4)
Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Michael Potter, M.D.
Program: Immunology, Support Site Visit Date: None (The Project Offi-
Technical Review Group: Tumor Immunology Review Group cer visits the site)
Relevance Review Group: DCBD Contract Program Review Committee
FY75 Funds: $196,691

CONTRACT RESEARCH SUMMARY

Title: Studies of the Immune Response of Mice and Rats to Tumor Antigens

Principal Investigator:	Dr. William Wallen
Name/Address	Litton Bionetics, Inc.
Performing	5510 Nicholson Lane
Organization:	Kensington, Maryland 20795

Contract Number: N01-CB-53889
Starting Date: April 28, 1975 Expiration Date: April 27, 1976

Goal: Study of immune responses of mice and rats to tumor-associated antigens. Studies on in vivo resistence to tumors and correlation with in vitro cellular and humoral immune responses.

Approach: The kinetics of the cellular and humoral immune responses to tumor-associated antigens of the following tumors will be studied: tumors induced by Gross and other murine leukemia viruses and murine sarcoma virus; methylcholanthrene-induced tumors. The relationship between the detected antigens and viral gene expression of fetal antigens will be extensively studied.

The specificity of each of the detected antigens in both the humoral and cell-mediated immune studies are being intensively studied by inhibition assays. The nature of the cells involved and the mechanism of cytotoxicity in the cell-mediated cytotoxicity assays are also being studied. A major emphasis is being placed on relating in vitro cell-mediated reactivity to in vivo protection against tumor growth by adoptive transfer experiment.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6, Approach 4)
Understanding the relationship of in vitro assays to host resistance to tumors is an essential element in properly using these assays to monitor response to immunotherapy.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology, Support Site Visit Date: None
Technical Review Group: Ad Hoc Review Committee
Relevance Review Group: Staff
FY75 Funds: $182,134

CONTRACT RESEARCH SUMMARY

Title: Immunodiagnostic Testing, Antibody Measurement and Fractionation of Serum and Urine

Principal Investigator: Dr. Ram Das
Name/Address Meloy Laboratories, Inc.
Performing Springfield, Virginia 22151
Organization:

Contract Number: N01-CB-33875
Starting Date: November 26, 1965 Expiration Date: April 10, 1975

Goal: To use quantitative and qualitative immunochemical techniques for improved diagnosis of cancer and to evaluate humoral immune responsiveness of patients with cancer.

Approach: Qualitative and quantitative immunochemical techniques are used for the diagnosis of multiple myeloma, Waldenstrom's macroglobulinemia, agammaglobulinemia and other diseases with immunoglobulin disorders. The same techniques are used to evaluate carcinoembryonic antigen and α-fetoprotein in the serum of patients with a variety of malignancies. Appropriate reagents for the immunoglobulin and carcino-fetal antigen detections are prepared and evaluated. The Immunoglobulin Reference Center serves as a distribution outlet for reference material for human serum immunoglobulin quantitation. In cooperation with the World Health Organization, prepares and distributes international immunoglobulin reference serums. Finally, the Reference Center serves as a focus for the education of investigators and their technicians in the techniques and interpretations of various immunodiagnostic procedures.

Progress: CEA assays were performed on sera supplied from various clinical trials. A cooperative study indicated that the CEA assay as performed at the Reference Center was as accurate and reliable as other types of assays in distinguishing elevated from "normal" CEA levels.

Alpha fetoprotein and human chorionic gonadotropin assays have been used to follow metastatic testicular disease during drug therapy. In all cases where there was a persistent elevated marker, there was a clinical recurrence of the disease within 14 months. Alpha fetoprotein levels were elevated in over 75% of a large series of patients with hepatic cellular carcinoma and testicular tumors, and from 5-22% of patients with pancreatic, gastric, colon and pulmonary and less than 0.4% of patients with benign nonhepatic diseases.

In addition, assays of the presence of antibody to eight different antigens were determined on approximately 500 sera obtained from patients with malignant or immunodeficient disease.

Class specific goat antisera have been raised against human heavy chain classes alpha, gamma, delta, and mu and light chain kappa. These are currently being conjugated to fluorescent isomes for use in typing human lymphocytes.

Significance for Cancer Research (NCP Objective 5 Approach 4)

A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after surgery.

Project Officer: W. D. Terry, M.D., M. Blaese, M.D., T.A. Waldmann, M.D., and H. B. Dickler, M.D.

Program: Immunology, Support Site Visit Date: November 19, 1973
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: DCBD Contract Program Review Committee
FY 75 Funds: $97,640 - supplement

CONTRACT RESEARCH SUMMARY

Title: NCI Human Histocompatibility Typing Center

Principal Investigator:	Dr. R. J. Trapani
Name/Address Performing Organization:	Microbiological Associates, Inc. 4733 Bethesda Avenue Bethesda, Maryland 20014

Contract Number: NCI-CB-43915
Starting Date: June 16, 1969 Expiration Date: March 15, 1975

Goal: To assay solubilized HL-A alloantigens provided by the Project Officers and to study HL-A and other cell surface antigens on normal and neoplastic cells and their subcellular fractions.

Approach: The assay for solubilized HL-A alloantigen utilizes a cytotoxic inhibition assay. Target cells bearing the HL-A alloantigenic determinants to be analyzed are labeled with ^{51}Cr. The solubilized materials are incubated with the solubilized antigenic components and the inhibition of reaction of these antisera to the target cell demonstrates the presence of this antigenic material. Various serological tests, including cytotoxicity, C' fixation, and agglutination are used to assay HL-A and other antigens and human white blood cells and tissue culture cells both normal and malignant. Lymphocytes of patients with various malignant diseases are assayed to determine associations between these antigens and disease. Subcellular particles of human lymphoid cells are studied for presence of HL-A antigens.

Progress: The contractor has performed assays on HL-A and other cell surface antigens on material provided by the Project Officer. Large numbers of sera from patients immunized with allogeneic leukemic cells were screened for antibody to HL-A or acute leukemia antigens. These patients developed antibody to HL-A alloantigens but not to leukemia associated antigens. Antisera to subcellular membrane components have been produced by the contractor. These sera appear to be detecting non-HL-A allotypic antigens.

In addition, a study of HL-A antigen frequencies in selected diseases, especially malignancies, has been undertaken. Of major importance is the finding that lung cancer patients possessing either HL-A5 or W-19 had a 60% chance of remaining disease free for at least one year following therapy. Those patients not possessing either of these antigens had only a 15% chance of remaining disease free. A possible reason for this may be an HL-A associated antitumor immune response gene.

Significance for Cancer Research (NCP Objective 3, Approach 1, 5)
Development of methodologies in the identification of isolated cell surface antigens is a necessary prerequisite to the identification of tumor specific associated antigens.

Project Officer: Dean L. Mann, M.D., G. Nicholas Rogentine, Jr., M.D.
Program: Immunology, Support Site Visit Date: July 1973
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY75 Funds: $17,551(supplement) -This contract will terminate March 15, 1975.

CONTRACT RESEARCH SUMMARY

Title: Preparation of Reagent Antisera and Antigens

Principal Investigator: Dr. Howard M. Grey
Name/Address National Jewish Hospital and Research Center
Performing 3800 East Colfax
Organization: Denver, Colorado 80206

Contract Number: NCI-CB-53912-31 (Selective continuation of N01-CB-33875)
Starting Date: May 1, 1975 (estimate) **Expiration Date:**

Goal: Preparation of monospecific fluorescent-isomer conjugated IgG antibodies and $F(ab')_2$ fragments thereof to all heavy and light chain classes of human and mouse immunoglobulin and human B and T lymphocytes.

Approach: All classes of human and mouse heavy and light chains will be purified by immunochemical techniques. These will be used to immunize animals and will be conjugated to Sepharose beads. The resulting antisera will be absorbed with appropriate conjugated beads to render them monospecific. The IgG fractions of such antisera will be isolated and conjugated to fluorescent isomers. A portion will be enzyme digested to make $F(ab')_2$ fragments. A variety of cell types will be used for both immunization and absorption to make human B and T cell specific antisera. The antibodies will then be further processed as for the anti-Ig reagents.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3, Approach 5)
To provide specialized antisera required for cell surface studies important in the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Howard B. Dickler, M.D., Warren Strober, M.D.
Program: Immunology, Support **Site Visit Date:** N/A
Technical Review Group: Ad Hoc Review Committee
Relevance Review Group: Staff
FY75 Funds: $68,976 (estimate)

CONTRACT RESEARCH SUMMARY

Title: Production of Human Lymphoid Cells in Culture

Principal Investigator:	Dr. Sami Mayyasi
Name/Address	Pfizer, Inc.
Performing	199 Maywood Avenue
Organization:	Maywood, New Jersey 07607

Contract Number: N01-CB-12078
Starting Date: January 1, 1971 Expiration Date: December 31, 1975

Goal: To grow and produce large quantities of human lymphoid cells in long-term tissue culture and to grow the Raji cell line in human serum for immunization of acute leukemia patients.

Approach: Several human lymphoid tissue culture cell lines which bear different HL-A alloantigens are produced in large quantities and are isolated, concentrated, and shipped to the Project Officer for subsequent use in the isolation and characterization of the HL-A alloantigens. One of the cell lines, Raji, is being grown in quantities sufficient to initiate an immunization program in acute leukemia in humans. This cell line, unlike the other cell lines, is grown in normal human serum to eliminate the possibility of immunization with the fetal bovine serum foreign protein.

Progress: During the last contract period, 7254 grams of lymphoblastoid cells were supplied to the Project Officer. Over 6 kg of the cells were human; the remainder were murine. Raji, IM-1, and 4265 human lymphoblastoid cell lines grown in fetal calf serum were each produced at the rate of 550 per month. The murine cell lines EL-4, L-1210, and S-49 were cultured to yield a total of 1.171 g. Membrane obtained from human lymphoblastoid cell lines totaling 299.8 were produced and shipped. Pilot studies were conducted to determine optimum procedures for labeling human cells with 3H amino acids and murine cells with 3H arginine.

Significance for Cancer Research (NCP Objective 3, Approach 5)
Provides prototype technique for the isolation of tumor specific or tumor associated antigens. Raji cell line has been used for immunotherapy in acute leukemia.

Project Officer: Dean L. Mann, M.D.
Program: Immunology, Support Site Visit Date: June 1972
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: Ad Hoc DCBD Contract Program Review Committee
FY75 Funds: $243,226

ANNUAL REPORT

THE BREAST CANCER PROGRAM COORDINATING BRANCH AND THE BREAST CANCER TASK FORCE PROGRAM

July 1, 1974 - June 30, 1975

INTRODUCTION AND SUMMARY

The Breast Cancer Task Force in its fourth year of operation presented for the first time an overview of its activities entitled "A Report to the Profession from the Breast Cancer Task Force." The intent was to inform the scientific as well as the lay community of the programs and accomplishments of its five Committees - Diagnosis, Epidemiology, Experimental Biology, Treatment and Virology. A transcript of the presentations has been published and made available to numerous individuals in this country and abroad.

The Breast Cancer Treatment Committee also had a "first of a kind" meeting. It brought together scientists from the United States and several other countries who are working on the estrogen receptor technique for predicting the preferable mode of treatment, endocrine or chemotherapeutic, for the patient with breast cancer. Additional details on the meeting are presented in the individual report of the Treatment Program, and a book of the proceedings has been published.

Arrangements for the meetings described above and assistance in the preparations of the publications are included in the responsibilities of the Breast Cancer Program Coordinating Branch, the supportive staff for four of the five Committees which form the Task Force. The Breast Cancer Virus Working Segment collaborates closely with the Task Force although it is a part of the Division of Cancer Cause and Prevention.

In the course of the past year the BCTF contract program has consisted of approximately 70 projects. The technical review Committees and the Branch Staff have the responsibility of reviewing and evaluating the ongoing activities of these contracts. During FY-1975 they have approved the renewal of the majority of them while terminating others. Three of the four Committees advertised seven new projects during the past year which resulted in the funding of 10 new contracts. By the end of June 1975 the Program is expected to include 78-80 projects with a total funding of approximately $7.5 million. Plans for new projects for the Program have been formulated by the respective Committees for advertising in FY-1976.

The major change in the Program of the Task Force in FY-1975 has been in the chairmanship. The Chairman of the past four years resigned from the Cancer Institute. Also, the Chief of the Breast Cancer Program Coordinating Branch retired after 30 years of government service and has been replaced. The growth and productivity of the Program in breast cancer have been directed and nutured by the devoted efforts and interests of these two leaders, Drs. Nathaniel Berlin and Erwin P. Vollmer.

THE BREAST CANCER DIAGNOSIS PROGRAM

In its initial phases the Breast Cancer Diagnosis Program was designed with three broad objectives: development of diagnostic, clinical and biological classification criteria, development and testing of screening techniques, and the subsequent application and evaluation of these screening techniques on large sample populations.

In fulfillment of these goals, the Committee has been devoting its efforts in these major research areas: radiology-detection, immunology and diagnosis-prognosis.

With the establishment and operation of the Breast Cancer Demonstration Projects throughout the country, research in radiologic detection is assuming vital importance. There is an increasing awareness of radiation hazards and concommitantly a stronger demand for imaging technology which will reduce radiation dosage substantially without loss of resolution. To this end, three contractors have been funded for development and refinement of physical instrumentation for detection. One is building an ultrasonic scanner which will soon be available for clinical testing; another is attempting to optimize an electronic mammography technique; and, the third investigator is designing an experimental multi-element infrared scanning system to be tested clinically for use in a mass screening program for breast cancer. A study involving work at three institutions is also in progress to compare the relative merits of xeroradiography versus conventional film mammography to delineate differences between them which may determine the use of one modality over the other. Much more emphasis will be devoted during this coming year to development of research protocols for ascertainment of optimal frequency of screening and reduction of radiation dosage.

The Breast Cancer Diagnosis immunology program began in FY-1972 when five contracts were initially negotiated. A threefold thrust was envisioned. The first was to insure a supply of human breast cancer tissue for immunodiagnostic studies. One contract has been ongoing for three years for this purpose. Secondly, a necessity to culture and maintain long-term human breast cancer lines was recognized. Two investigators have become involved in the growth and isolation of human neoplastic mammary epithelium, one from solid tumor material, the other from pleural effusions. The former contractor will be terminated during FY-1976. The latter has established six tumor cell lines from pleural effusions on which proper characterization to determine their neoplastic nature is being carried out by chromosomal analysis, electron microscopy and production of tumors in immunodeficient rodents. Precise characterization of cytokinetic, immunologic and biochemical properties of these cell lines is now under way. The third objective, to develop immunodiagnostic assays for human breast cancer associated antigens, was initiated with two contractors. One was terminated in FY-1974, the other is actively engaged in the purification of antigens from human mammary carcinoma and in definition of their immunochemical properties, their distribution and potential value for serological diagnosis of high risk populations. An antigen recently detected in breast tumors was identified as ferritin. A double-antibody RIA developed for ferritin revealed elevated serum ferritin levels in 33 percent of preoperative patients with newly diagnosed breast cancer and in 68 percent with metastatic breast tumors. Studies have also been initiated to identify antigens that elicit cell-mediated immunity to breast tumors. Preliminary work appears to demonstrate the presence of cell-mediated immunity in breast cancer patients to antigenic determinants present on both autologous and allogeneic breast tumors. Greater input by the Committee into the immunologic aspects of breast cancer diagnosis is being considered for the next fiscal year.

In the diagnosis-prognosis area, a three-year collaborative investigation to assess the prognostic significance of combinations of various clinical, pathologic and urinary steroid variables in patients with breast cancer treated by radical mastectomy is nearing completion. The data are now undergoing statistical evaluation. Two other case-control studies are concerned with determination of steroid sulphate levels; one in plasma of premenopausal and postmenopausal women utilizing radioimmunoassay techniques, the other in 24-hour urines by gas chromatography-mass spectrometry methodology. These steroids are being measured as possible biochemical markers for the identification of populations at high risk. The role of steroidal epoxides in the etiology of breast cancer is also being probed. In another effort to define early diagnostic criteria utilizing other modalities, two collaborating investigators are actively involved in securing samples of breast secretions by the Sartorius pump for biochemical, virological, immunological and cytological evaluation. In addition, a project to clarify the association between fibrocystic disease and breast cancer is entering its second year.

To further expand the breast cancer diagnostic effort, the Committee issued three RFP's in FY-1975. One was concerned with the biochemical analysis of breast cyst fluid and its correlation with breast cancer. Nine proposals were received in this category and one was accepted for funding. An RFP issued for mammographic detection of early breast lesions resulted in no awards. The third RFP was directed at optimization and standardization of 70 mm photo-mammography and its clinical comparison with conventional mammography. The Reviewing Committee recommended substantial revision of the technical and experimental design aspects of one pending proposal prior to its subsequent reevaluation.

During FY-1975 the Breast Cancer Diagnosis Committee has terminated eight contracts, renewed fourteen and initiated two new ones.

BREAST CANCER EPIDEMIOLOGY PROGRAM

The major emphasis of the Breast Cancer Epidemiology Program in FY-1974 has been on familial and genetic studies in breast cancer. The protocols for the new contracts which were initiated in this area emanated from a "Genetics and Breast Cancer" workshop held in late 1973 which was designed to develop specific research ideas for genetic and pathophysiologic studies of familial breast cancer. At that conference it was decided that members of families exhibiting familial concentrations of breast cancer should be investigated for genetic marker distributions, anthropomorphic constitution, endocrinological, virological and immunological characteristics. A precise characterization of the spectrum of mammary neoplasms observed within these high risk groups was also recommended.

Two years ago in an effort to stimulate family and pedigree studies, the Epidemiology Committee funded a resource consisting of some 80 breast cancer-prone families, thus making available to collaborating investigators a variety of biological specimens for medical genetic studies. Last year as a result of the workshop the decision was made to initiate two new contracts in familial studies utilizing this resource. One contractor is concerned with determination of a wide range of steroid and peptide reproductive hormone levels in blood and urine of premenopausal females of these high risk breast cancer families as well as those of female members of low risk families. The objective is to determine if a correlation exists between endocrine parameters and a familial susceptibility to breast cancer. The other contractor is investigating histocompatibility antigens (HL-A) and other genetic polymorphisms such as the blood groups (ABO, Rh, MN, Duffy, Lewis, P, K, Fy^a, Jk^a); red blood cell enzymes (acid phosphatase, phosphoglucomutase 1, adenylate kinase, esterase D) and serum proteins (haptoglobin α-chain, $alpha_1$-antitrypsin, group specific component) in these high and low risk breast cancer families. Pursuing the genetic polymorphisms studies further, a third investigator is using his own resource of breast cancer families. The high risk group in this study is composed of families of: a) patients whose mothers had breast cancer; and b) sisters of patients with at least a 30 percent probability of developing the disease by age 40. The disease in these families, in addition to developing early in life, develops in both breasts more frequently than expected. In addition to a search for genetic markers, chromosomal abnormalities and/or variants are being studied.

A group in Amsterdam is also involved in studying linkage of breast cancer with genetic markers such as HL-A haplotypes, blood groups, immunoglobulins, red cell isoenzymes and chromosomal polymorphisms. A histological classification of all tumor biopsies in their breast cancer-prone family patients is documented. Preliminary results show a high frequency of medullary carcinoma and a marked lymphocytic infiltration in the majority of tumors investigated within these families.

A fifth contract concerned with the study of familiality of breast cancer is funded in Iceland where such unique facilities as a nationwide cancer registry, a computer-based National Roster and a family linkage file are available. Early data confirm increase in risk factor of breast cancer in Iceland with increase in age at first birth.

The findings that variability in aryl hydrocarbon hydroxylase (AHH) inducibility may be under genetic control involving a single autosomal locus, and that patients with histologically confirmed bronchogenic carcinoma fall into the high or intermediate AHH inducibility range led the Committee to support a project to study the relationship of AHH inducibility to breast cancer. However, after a year of repeated attempts to increase the reproducibility of the assay it was decided to terminate this case-control study until a more productive assay could be developed.

Much interest has also been evinced in investigating the part that hormonal factors may play in mammary carcinogenesis. Several studies have been reported linking low estriol and low etiocholanolone ratios to breast cancer. A project was initiated last year to delineate the normal dynamics of estriol production and metabolism in relation to high and low excretors of estriol in order to determine the physiological basis for the reported differences between high and low breast cancer-prone individuals. Preliminary data indicate that androstenedione may be a significant source of estriol at the follicular and luteal phases of the menstrual cycle in women with high Estriol/Estradiol + Estrone ($E_3/E_2 + E_1$) ratios.

The role of exogenous factors in breast cancer etiology and pathogenesis has always been a subject of much concern to the Committee. A case-control study is in progress to investigate the part that obesity may play in breast cancer etiology. Data are being collected in the Netherlands on body weight and height to determine if these anthropometric measurements have an effect on the level and shape of the age-specific incidence curves for breast cancer in that country. To further expand the Epidemiology program in this area, two requests for proposals (RFP's) were advertised this year. One RFP designed to investigate the effect of non-contraceptive exogenous estrogens on the occurrence and natural history of breast cancer resulted in seven responses. Two proposals will be funded under this procurement. Another RFP, to investigate the possible correlation between dietary factors and epidemiological characteristics of breast cancer brought nine responses. Two investigators will be awarded contracts by June 30, 1975 for this study.

The third RFP issued this year, to which there were 13 responders, was aimed at initiating studies to seek correlations between morphologic and epidemiologic characteristics of human mammary carcinoma. Several attempts in past years to find worthwhile studies under a similar RFP had proved unsuccessful. This year a Swedish investigator using an innovative approach to improving the histopathologic classification of breast cancer by computer imaging has been funded. If this proves successful, correlations with established epidemiologic risk factors will be sought. Another response to this RFP linking morphological subentities to familial history of breast cancer is being supported.

A two-year project seeking a correlation between degree of duct atypia (using the Black-Chabon grading system) and subsequent development of breast cancer is being brought to completion. The report is being readied for publication.

In its total effort during FY-1975, the Epidemiology Committee has terminated two projects, renewed eight and initiated six new contracts.

BREAST CANCER EXPERIMENTAL BIOLOGY PROGRAM

One of the major goals of this Committee, since its inception three years ago, has been to improve the _in vitro_ cultivation of mammary cells with the specific objective of making human mammary cells, normal and neoplastic, available for general investigational use. Dr. Edwin Gaffney has succeeded in cultivating from human milk of a normally lactating woman an epithelial cell population which grows with a doubling time of about two days and a chromosomal mode of 66. There are no signs of neoplasia in the donor gland. Normally, epithelial cells isolated from human milk survive in culture for several days but do not multiply. The Gaffney line (HBL-100) apparently has some peculiar characteristics which permit growth where other identical epithelial cells fail to do so. These characteristics may limit the normality of this cell population however this remains to be determined. To our knowledge, the HBL-100 cells in culture are the closest to a normal, human epithelial population of mammary origin that exists. Thus, within these limits one of the important goals of the program has been reached.

Two other important results have been obtained in the area of cell cultivation: 1) The isolation by Dr. William McLimans of a rat cell population, which he calls steatotic, to emphasize the ability to produce milk fat in culture. This population represents an important tool for future work on differentiation and growth of mammary cells. 2) The observations by Drs. Elinor Mills and Joel Elias of a pattern in surface microvilli of human breast cancer cells, via scanning electron microscopy (SEM), may be pathognomonic of neoplastic transformation and may offer a new parameter in assessing the preneoplastic nature of mammary lesions.

The contract project of Dr. Gaffney was expanded during the past year in order to include a study of milk samples of normal, lactating women. Attempts are made to establish epithelial cultures from the milk samples and to study the cells for preneoplastic or neoplastic characteristics. The goal is to determine whether this procedure will contribute to early diagnosis or a potential lead as to high risk of breast cancer. Drs. Elias and Mills reorganized their contract program in order to increase the number of human samples examined by SEM. This is an attempt to determine the importance of their findings for pathological diagnosis as quickly as possible.

Importance of well characterized human breast cell lines, normal and abnormal, to many investigators in the Breast Cancer Task Force program prompted an addition of a cell line bank to the existing animal mammary tumor bank service contract at Mason Research Institute. Three such human lines, including the Gaffney HBL-100, are now in the bank and are being produced in sufficient quantity to provide investigators with the required material for characterization. A major effort of this contract is to provide a quality control of the material released, in particular, absence of microorganisms and PPLO, definition of optimal growth conditions, unique biological characteristics, etc.

A second major objective of the program has been to find markers which permit the recognition of mammary cells in culture when the complete functional differentiation (production of casein and/or lactalbumin) is not evident. Four contracts in this area have been funded during the past three years. Two of these are completing their terminal year, the other two projects are into their second year. One is developing ways of identifying antigenic markers on the cells and the other is looking for characteristic markers by analyzing isoenzyme patterns of G6PD and LDH. Definitive results are not yet available.

Another contractor is bridging between problems related to mammary cell recognition and hormone responsiveness of mammary cells. The laboratory has developed and carried to a high degree of refinement the technique of fluorescent polarization as a tool for quantifying various types of macromolecular binding. The project has the major objectives of finding whether this experimental tool will demonstrate that the mammary epithelial cell has specific receptor proteins for each hormone able to elicit a response.

Several approaches are being investigated in relation to the major question of hormone responsiveness of mammary tissue. The best known of the approaches at the present time, and the one which has produced definitive results applicable to clinical practice, is the study of estrogen binding proteins. Results obtained by Dr. William McGuire have demonstrated that a carcinoma with less than 3 f.moles/mg. cytosol protein has little or no chance of responding to hormonal therapy by growth arrest or regression. Thus, decisions on the therapy of mammary carcinoma patients after surgery must take into consideration the level of estrogen binding proteins of the tumor. The finding of Dr. Eugene DeSombre of a peroxidase positive reaction, inducible in hormone responsive but not in autonomous mammary tumors, and detectable on histological sections could dramatically simplify the diagnostic procedures if proven as reliable as the estrogen binding assay.

Another approach to hormone responsiveness is along the lines of organogenic development. The role of androgens and mesenchymal cells in controlling whether a "male" or "female" type of gland develops is under investigation. Because this work may not give the complete picture at the organ level another contractor is studying androgens, or lack thereof, in neonatal imprinting of the hypophyseal-hypothalamic axis as male or female type. Testicular feminization mutants and androgen-responsive mammary tumors are used in this work.

Two other contractors are looking at hormone responsiveness at the level of chromatin structure. Whether or not a pattern of genes is expressed, as a consequence of hormonal stimulation, will depend on the structure of the portion of chromatin containing the genes. This structure will, in turn, depend upon the presence and physical orientation of particular histones and acidic chromosomal proteins. These two investigators, at separate institutions, are trying to characterize the program of events related to histone and acidic proteins during chromatin formation in normal and neoplastic cells, and then relate this program to hormone responsiveness of the cell population.

In addition to the study referred to above which is concerned with the imprinting of the male-female characteristics of hypophyseal-hypothalamic axis during ontogenesis, two other projects are investigating hormone responsiveness through the relationship(s) of the hypophysis and mammary tissue. One contractor is studying the possible effects of the neoplastic mammary cells on the hypophysis and the other is concerned chiefly with prolactin receptors of mammary cells and the possible impact that this presence may have on the prognosis of the tumor growth. The Committee has indicated that more work should be done on the role of prolactin in mammary tumor growth, e.g., which portion of the prolactin molecule is needed for binding to cell surface sites and whether the binding site and hormonal properties belong to the same portion of the molecule.

A new project on hormone responsiveness of mammary cells, approved for funding this fiscal year, is aimed at the isolation and characterization of steroid binding proteins, estradiol in particular, from human milk. Its major objective is the use of milk as an efficient source of these proteins which can then be utilized for assessing their biological activity.

In the broad area of mammary tumor pathophysiology three contract projects are ongoing. Two of these are closely related and one of them could not have been initiated had not the other been highly successful in improving the growth of nude-athymic mice. Dr. Beppino Giovanella has increased the nude mouse colony to the point of having 1,500 to 1,600 on experiment at any given time and has supplied the other contractor with breeding stock for his project. In addition to improving the health and thereby the longevity of the nude-athymics Dr. Giovanella transplants human breast cancer specimens (from primary and metastatic tissue) into these hosts. Also, human cell lines of breast carcinomas have been grown successfully in the nudes, these tumors are transplantable, fast growing, and they metastasize. Thus, an important tool for the chemotherapist has been readied. The related project is undertaking the transplantation of preneoplastic lesions of human breast tissue into the cleared fat pads of the nude-athymic mice and the growth potential of these lesions is studied in detail.

The third pathophysiology project is investigating the peculiar characteristics of hyperplastic alveolar nodules (HAN) of rat mammary glands. These lesions have levels of estrogen binding lower than the normal gland and mammary tumors, and insulin + prolactin + hydrocortisone will induce casein production in HAN. The preneoplastic nature of the HAN is being characterized.

The role of the stroma in mammary tumor growth and regression is being investigated in two projects, one was initiated during the past year and the other was approved for funding this fiscal year. In the ongoing contract studies on the interrelations between fibroblasts of the fat pad and epithelial mammary cells are underway. The new project involves the synthesis, distribution and turnover of glucosoaminoglycans of the basal membrane. The major objective is to establish the role of this specific component of the stroma in post-natal mammary morphogenesis and tumor invasion. Members of the Committee believe additional work in the area of stromal epithelium interactions is required.

Influencing growth or any other biological characteristics of neoplastic mammary cells is being investigated in one contract initiated during the past year and one approved for funding this year. With the ongoing project the transfer of DNA given as free compound or complexed with proteins, or as contained in organelles (mitochondria, metaphase chromosomes, spermatozoa) is being studied. The other project will follow a similar approach but using mammary tumor virus (MTV) infected cells.

Another major area of investigation of the program of the Committee is that of conditions related to unrestrained growth. One contractor is attempting to purify serum factor(s) involved in the initiation of DNA synthesis in cultures of mammary epithelium. To date progress with the project has been undesirably slow. Two other projects involve work on the role of cyclic AMP (cAMP) in mammary tissue growth and neoplasia, one contractor is concerned with the isolation and characterization of the receptor-adenylcyclase complex, cAMP plus receptor protein, and phosphodiesterase systems of the mammary gland during development and neoplastic transformation. The other study of cAMP is designed to attempt to correlate rates of mammary tumor growth with quantities and activities of enzyme responsible for cAMP synthesis and degradation. Both of these studies as well as the ones to be discussed below, should profit from the availability of the human breast cell lines. Like the two projects on cAMP there are also two on the role of prostaglandins (PG) in mammary tumor growth. One of these is for the study of human tumors, to relate their growth rate with PG levels and conditions altering these levels. The other investigator is working chiefly with rat and mouse tumor systems and is trying to obtain enzymatic preparations able to synthesize PG, and is studying the in vitro and in vivo conditions affecting PG synthesis.

In the area of mammary tumor regression four projects have been funded since the beginning of the program, one of these is terminating this year. It has not been totally unproductive, however, the data obtained to date on the involution process of the lactating gland failed to be indicative of making an important contribution in this area. The other three projects are directed to: 1) the study of the interaction between lymphocytes and mammary epithelium, normal and neoplastic; 2) the effects of mammary tumors on specific and non-specific inhibition of cellular immunity; and, 3) the possibility of influencing mammary cell survival by reducing the host blood level of phenylalanine, phenylalanine ammonia-lyase (PAL) is being purified and is administered to tumor bearing mice (C_3H) to determine whether the agent can effectively reduce phenylalanine levels. Although this latter contract was initiated only in June 1975, the investigator has found that PAL will inhibit mammary tumor growth in vivo. These early results remain to be found reproducible and many other experiments are required before human evaluation is considered.

Three projects are designed to study mammary tumor metastasis. Two of the projects are attempting to devise methods for isolation and characterization of cytoplasmic membranes from mammary epithelial cells. One investigator is approaching the problem by producing unique organic chemicals, the results are incomplete but they appear promising. The other investigators, two on one contract, are employing biochemical techniques; again, the results appear promising. Under the third project metastasizing and non-metastasizing mammary carcinomas of inbred rat strains have been developed and conditions which may influence cell dissemination in the host are being determined. This investigator has several publications on his work in press and he has supplied other investigators with the metastasizing tumors for their respective projects. Metastasis is another area of mammary tumor biology which the Committee believes should be expanded.

No new projects were advertised by the Experimental Biology Committee during this fiscal year. However three new contracts were funded, these had been approved by the Committee the previous year depending on the successful outcome of project site visits. At the beginning of the new fiscal year this Committee will have a total of 33 contracts, 30 renewals and three new ones. Six contracts were terminated.

BREAST CANCER TREATMENT PROGRAM

The primary objective of the treatment program is to improve the cure rate for patients with breast cancer. The activities sponsored by the Treatment Committee have emphasized four areas of study; 1) cell kinetics, a search for methods of measuring tumor cell growth characteristics predictive of clinical behavior; 2) animal tumor models, the study of the effects of experimental treatment programs on animal mammary tumors and their hosts; 3) tumor cell markers, the investigation of biochemical substances found in tumors or in body fluids as indicators of the presence of tumor or of response of tumor to treatment and, 4) clinical trials, a series of controlled prospective studies designed to evaluate the effectiveness of treatment for patients with all stages of breast cancer.

In the area of cell kinetics, four projects were undertaken during the 1973 and 1974 fiscal years and three are continuing. One of these has successfully developed procedures which obtain single cell suspensions from solid tumors. Subsequent studies will utilize these suspensions for rapid automated studies of the influences of treatment upon cell characteristics. Another project utilizes the primer available DNA polymerase (PDP) assay to determine cell cycle parameters. Individual cytokinetic results are now obtainable in seven days in the C3H mouse mammary tumor. Similar experiments on human breast tissues are underway. The third ongoing project defines cell replication patterns of human tumor cells in vivo and in vitro. Alterations in labeling patterns and DNA synthesis will be evaluated in serial biopsy specimens obtained before and during treatment. The last of the four contracts completed its work near the end of FY-1975. It had been set up to evaluate the relationship between the labeling index of tumor cells and the subsequent response to therapy. Testing of several methods failed to produce consistent data and it was decided to curtail this approach.

The only contract involving animal tumor models provides tumor-bearing animals for cell kinetic studies and supplies tumor response and toxicity data to the clinical investigators. The most extensively used test system has been the transplantable metastasizing rat tumor, 13762 mammary adenocarcinoma. Experiments have involved single agent and multiple agent chemotherapy, immunological adjuvants, hormonal manipulations, surgery and radiotherapy. Data from these experiments are being utilized in the design of clinical studies.

In the tumor cell marker area of study, the Committee supports four projects. Two are investigative and combine methodology development with clinical assessment. The other two are support contracts and carry out specified laboratory determinations on material submitted by collaborating investigators whose protocols have been approved by the project officers. Estrogen receptor (ER) protein assays of human breast cancer tissues are carried out by one of the contract laboratories and radioimmunoassays of human serum for prolactin content by the other. The results of the assays will be examined for possible correlation with clinical responses. Dr. Dao at Roswell Park Memorial Institute is studying the estrogen sulfating enzymes in primary breast cancers to determine their value as predictors of the growth potential of the tumors and of clinical response to endocrine treatment, especially adrenalectomy. The currently most effective marker is the estrogen receptor. Dr. Elwood Jensen, a pioneer investigator,heads a project for further technical development and for analysis of clinical data from the assayed patients.

The Treatment Committee sponsored a Workshop on Estrogen Receptor Clinical Correlation in July 1974, which brought together the twelve biochemist-clinician investigative teams that have reported most widely about ER. Although assay techniques were discussed, the emphasis was on the clinical reports concerning 600 endocrine treatments in patients whose tumors had been assayed for ER. One hundred sixty-two of 298 treatments (54%) in patients with tumors considered ER positive had resulted in objective remissions, while only 19 of 282 (7%) had been successful when the tumors were ER negative. Eight of the groups availed themselves of the proffered services of an extramural review team. The clinical data from 436 of the 600 treatments were interpreted according to standard criteria for tumor response. The correlation between ER and clinical data in these 436 treatments was similar to the overall results reported above. The proceedings of the workshop were published in a hard bound volume, "Estrogen Receptors in Human Breast Cancer," edited by W. L. McGuire, P. P. Carbone and E. P. Vollmer.

Eight projects supported by this Committee deal with clinical trials. The National Surgical Adjuvant Breast Project completed entry of patients with Stage I or Stage II cancers into two studies and began accession into two new studies. Preliminary findings from one completed project indicate that radical mastectomy, total (simple) mastectomy plus radiotherapy, and total mastectomy alone are equivalent for the treatment of Stage I breast cancer. Long-term postmastectomy treatment with l-phenylalanine mustard (PAM) appears to reduce the incidence of early relapse in the patients who had 4 or more positive axillary nodes. The new studies are outgrowths of these; one compares the effectiveness of total mastectomy with lesser surgery for Stage I cancer and the other compares the original PAM regimen with a two-agent adjuvant treatment, PAM + 5-Fluorouracil. Four additional projects are testing combined modality treatments for Stage II cancers and utilize multiple chemical agents, immunostimulating materials, anti-estrogens and corticosteroids. Two projects are concerned with metastatic breast cancer; the first compares the effectiveness of oophorectomy alone with that of oophorectomy plus chemotherapy and the other compares estrogen therapy with estrogens plus chemotherapy. An additional treatment approach was sought by the RFP issued in FY-1975, "Suppression of Endocrine Function by Systemic Agents as Treatment of Human Breast Cancer." A project in this area was funded near the end of FY-1975. Its purpose is to compare the clinical effectiveness of aminoglutethimide with adrenalectomy and with oophorectomy and to elucidate the physiological mechanisms of hormonal suppression. These eight clinical projects have been chosen for sponsorship at least partially because they are complementary and supplementary to each other as well as to other well-designed studies supported by the National Cancer Institute.

FUTURE PROGRESS

Each Committee of the Task Force will continue to evaluate their respective programs in order to determine projects which are non-productive; seek new tasks that need to be undertaken; promote interaction between committees; and, encourage the publication of data from completed projects so that it will be made available quickly for physicians and basic scientists outside the program. Collaboration with other programs of the Cancer Institute which have a working interest in breast cancer, e.g., Breast Cancer Virus Working Segment and Cancer Control, has increased commendably in the past year. In the coming year an effort will be made to promote cooperation with the Immunology and the Carcinogenesis areas of NCI. The new Chairman of the Task Force and the new Chief of the Branch will continue to work toward achieving the goals of the "Detailed Research Logic Plan" as designed four years ago for the Breast Cancer Task Force program.

CONFERENCES AND WORKSHOPS

The following is a list of working conferences held under various Breast Cancer Task Force auspices since June 30, 1974.

July 18-19, 1974	"Workshop in Estrogen Receptor Clinical Correlations." Sponsored by the Treatment Committee.
September 30, 1974	"Report to the Profession on Breast Cancer" (Bethesda). Sponsored by the Breast Cancer Task Force.
February 10-12, 1975	"Annual Program Review Conference, Breast Cancer Task Force" (San Antonio). Sponsored by the BCTF Steering Committee.

PUBLICATIONS

Levine, B., Tarwater, E. and Vollmer, E. P. (Eds.): Report to the Profession. Breast Cancer. Bethesda, Md. Breast Cancer Program Coordinating Branch, 1975, 202 pp.

McGuire, W. L., Carbone, P. P. and Vollmer, E. P. (Eds.): Estrogen Receptors in Human Breast Cancer. New York, Raven Press, 1975, 298 pp.

CONTRACT RESEARCH SUMMARY

Title: Ultrasound Mammography

Principal Investigator: Gilbert Baum, M.D.
Name/ Address Albert Einstein College of Medicine
Performing Bronx, New York 10461
Organization:

Contract Number: N01-CB-23872
Starting Date: June 20, 1972 Expiration Date: June 13, 1976
Goal: To develop the criteria for detection and for the differential diagnosis of breast cancer employing ultrasound, a non-invasive, painless and harmless modality.

Approach: Patients who are to undergo breast surgery are referred into the study on a random basis from breast clinics and surgeons. Scanning is performed on each breast, where the unaffected breast is used as the control. Scanning is begun at the top of the breast and continues down in vertical 3 mm. steps. The ultrasonograms are recorded on 35 mm. half frame film (black and white) and the films from both breasts are simultaneously projected for comparison. Additional film analyses are conducted using an isophotodensitometer, which converts the grey scale of the films into specific colors, in which each color indicates a specific range of amplitude. Finally the ultrasound, clinical and x-ray diagnoses are compared against the pathological report and statistical evaluations are conducted.

Progress: To date, we have examined about 100 patients 53 of which underwent surgery. Our clinical results are as follows: rate of agreement with pathology is 86.7%; false positive rate is 18.5%; false negative rate is 7.5%. Our goals are to refine and simplify detection and interpretation of tissue signatures which signify early malignant change and, ultimately, to be able to perform breast cancer examinations on a screening basis. To accomplish this end, a scanner specifically designed for ultrasound mammography is nearing completion. Clinical evaluation will be begun this coming fiscal year.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/26/75
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $158,633

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator:	George Blumenschein, M.D., Relda Cailleau, Ph.D.
Name/Address	M.D. Anderson Hospital & Tumor Institute
Performing	University of Texas
Organization:	Houston, Texas 77025

Contract Number: NO1-CB-23869
Starting Date: June 1, 1972 Expiration Date: August 31, 1975

Goal: To establish tumor cell lines from solid tumors or pleural effusions from breast cancer patients. To supply these cells, effusions and/or blood samples to collaborators for immunodiagnostic assays or other studies. To characterize the lines we have established by chromosomal, biochemical or immunological methods.

Approach: Cells from fresh pleural effusions are concentrated by centrifugation, hemolyzed, neutralized, and resuspended in various media. Aliquots are initially taken for chromosome analysis, staining and electron microscopic study. Primary cultures and established cell lines are frozen in glycerin or DMSO at -70^{o} or in liquid nitrogen for storage or later distribution.

Progress: Only temporary short-term cell growth has been obtained with the solid breast tumor specimens cultured (over 100 samples) but no tumor lines were established. Since January 1, 1973, we have been successful in establishing 7 lines from pleural effusions. From 3 of our patients sequential pleural effusions were obtained and yielded 4 and 6 and 2 sublines respectively, all of which resembled the original "parent" line. Chromosomal studies showed karyotypes distinctive for each line and some characteristic "extra-long" chromosomes were frequently seen. Electron microscope studies (Dr. Mackay) have shown all lines to be of epithelial and probably tumor origin. Inoculations into ALS and ATS-treated Balb/c mice (Dr. Maruyama) and nude mice (Dr. Giovanella) have produced tumors in 3 instances (to date) which histologically resemble the solid breast tumors of origin. Tests for G-6-PD indicate our lines are type B. Most of our solid tumor material is sent to our collaborator, Dr. Lasfargues, while blood samples and aliquots of pleural effusions are supplied to Dr. Grace Cannon. Our cell lines have been sent to the Breast Tumor Cell Bank and to Dr. Jorgen-Fogh of Sloan Kettering for storage and distribution.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Ronald B. Herberman, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 5/31/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography - A Comparative Study

Principal Investigator:	Gerald Dodd, M.D.
Name/Address	M.D. Anderson Hospital
Performing	Houston, Texas 77025
Organization:	

Contract Number: N01-CB-43993
Starting Date: June 28, 1974 Expiration Date: March 27, 1976

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: This project is being carried out among three institutions, University of Texas M.D. Anderson Hospital, Duke University Medical Center and Stella and Charles Guttman Breast Diagnostic Institute. Mammographic image formation and information transmitting characteristics of each process will be assessed by way of radiographic contrast, latitude, sensitivity and modulation transfer function. An advisory and monitoring body set up by the Diagnostic Radiology Committee of NCI will review the technical quality of the work performed by the participating institutions.

Progress: To date, a complete, practical clinic protocol has been established at M.D. Anderson Hospital to accept patients in the contract study period. All women over 40 years of age proceeding through the breast clinic are being included in the study period. One cephalo-caudal view of each breast is being obtained on the following mammographic x-ray units and recording media: 1) baseline non-screen film mammogram (Kodak-Type AA film) will be obtained with compression on a molybdenum anode, molybdenum filter mammographic unit (Siemens Mammomat); 2) low exposure, single-screen film mammograms (DuPont Lo-Dose System) will be obtained with compression on a molybdenum anode, molybdenum filter mammographic unit (Siemens Mammomat); 3) xeromammogram will be obtained with compression on a tungsten anode, modified Wolfe-cone mammographic unit. At the present time 100 patient studies have been completed according to the above protocol. Comparative images from other collaborating institutions are already circulating for interpretation. Contract images have been obtained with the fibril phantom and are being analyzed for differences in recording ability for each of the three systems. The microdensitometer has arrived at M.D. Anderson and is being used to scan edge images. Orders have been placed for the equipment to be used in the image research program. A purchase order for work on several aspects of the image research program to be accomplished at the University of Chicago has been sent.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/28/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $52,500 (nine months)

CONTRACT RESEARCH SUMMARY

Title: Pathology Laboratory for Cooperative Clinical Studies

Principal Investigator:	Gilbert Friedell, M.D.
Name/Address	St. Vincent Hospital
Performing	Worcester, Massachusetts 01610
Organization:	

Contract Number: N01-CB-80704
Starting Date: March 18, 1968 Expiration Date: April 16, 1976

Goal: To continue the operation of a central pathology laboratory as part of the Primary Therapy of Breast Cancer Study.

Approach: The objective of the above study is to find a correlation between clinical, pathologic and urinary steroid findings which would serve as prognostic indices in women with breast cancer. The central pathology laboratory was established for this study to achieve a standardization of sampling and preparative procedures for histopathologic studies and uniform systematic evaluation. Clinical material is being provided by the members of the Cooperative Breast Cancer Group.

Progress: The initial pathologic studies aimed at assessing the presence of metastases in regional lymph nodes, the presence of blood cell invasion and nuclear grade at the primary tumor site. The data is now being analyzed for correlation with clinical status and urinary steroid findings.

Significance for Cancer Research (NCP Objective 5B Approach 6)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $19,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: Raymond Fink, Ph.D.
Name/Address Performing Organization: Health Insurance Plan of Greater New York
New York, New York 10022

Contract Number: N01-CN-35021
Starting Date: June 28, 1973 Expiration Date: September 30, 1975

Goal: To evaluate the contribution thermography may make in increasing the effectiveness and/or efficiency of breast cancer screening programs.

Approach: 20,000 women will be screened by three methods: clinical examination, thermography and mammography, each modality being evaluated independently. To obtain the study population, a random sample of all women aged 45-65 belonging to specified medical groups of HIP will be selected. All findings will be reviewed by medical coordinator, who will be responsible for follow-up medical recommendations. This project is being run in conjunction with Thomas Jefferson University, N01-CN-35027.

Progress: Screening for breast cancer started during the first quarter of 1974. Through the end of September 1974, 13,805 women were contacted and invited to participate in the study. Of these, 5,378 or 39 percent completed the initial examination. As of October 31, 1974, 90 patients had completed diagnostic workup, of which 18 were diagnosed with malignant disease. Of the 18, 89 percent had positive clinical findings, 50 percent had a positive mammogram, and 33 percent had a positive thermogram. Approximately 25 percent of those examined have findings, thermographic, clinical or mammographic, that require follow-up in six months.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Harvey Geller
Program: Cancer Control Site Visit Date: March 24-25, 1975
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $1,121,352

CONTRACT RESEARCH SUMMARY

Title: Mass Screening for Breast Cancer by Electronic Infrared Pattern Recognition

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address Performing Organization: University of Oklahoma Health Sciences Center
Oklahoma City, Oklahoma 73190

Contract Number: N01-CB-43869
Starting Date: April 1, 1974 Expiration Date: Sept. 30, 1975
Goal: To design and clinically test a prototype system for mass thermographic screening of breast cancer and couple it with computer processing for automated evaluation.

Approach: This project is phase 1 of a planned 3-phase development program. The first phase would include: (1) construction of an improved instrument utilizing the unique serial scan multiple element developed by Honeywell; the instrument will be equipped for direct recording on a standard video recorder and conventional television display; (2) a program of clinical evaluation to assess the utility of the various features in the diagnosis of breast cancer and compare with results of other modalities; (3) definition of the system specifications for a mass screening prototype instrument, and (4) development of initial data base for automated screening evaluation.

Progress: Honeywell is currently assembling an infrared scanner using a multiple element mercury cadmium teluride detector. As soon as the instrument is available, the clinical portion of the project will be conducted in the University of Oklahoma Breast Cancer Screening Center.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/12/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'74 Funds: $234,000 (18 months)

CONTRACT RESEARCH SUMMARY

Title: Determinations of Steroid Sulfate Content in Blood to be Used as Diagnostic Parameters in Breast Cancer

Principal Investigator: Evan C. Horning, Ph.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas 77025
Organization:

Contract Number: N01-CB-43897
Starting Date: January 1, 1974 Expiration Date: December 31, 1975

Goal: To make a detailed comparison of steroid metabolites in urine in a search for possible diagnostic markers for breast cancer.

Approach: Urinary steroid metabolic profiles will be determined using gas chromatographic-mass spectrometric techniques on a group of 25 patients with breast cancer and compared with similar profiles for normal controls and for patients with benign lesions. These studies will be carried out with two 24-hour urine collections for breast cancer patients, for patients with benign lesions and for matched controls. Premenopausal and postmenopausal groups will be used. Another hypothesis to be tested is that breast cancer is caused by steroidal epoxides formed in breast tissue or through liver metabolism. This proposed work involves a study of urine of breast cancer patients for trace amounts of steroidal epoxides, and for possible precursors and metabolic products by means of gas phase analytical methods.

Progress: Technological improvements have been made in computer-based gas chromatographic analyses of human urinary steroids. Improved methods of computer-based analyses of GC-MS-COM repetitive scan data for steroid profiles have also been developed. Urinary steroid metabolic profiles from patients with benign breast lesions indicate that the steroid metabolism of these individuals is not normal. Breast cancer patients also have steroid profiles that are not normal. Some profiles resemble those of patients with benign lesions. The results suggest that it may be possible to define a high risk group.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 11/19/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $110,000

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator: Etienne Lasfargues, D.V.M.
Name/Address Institute for Medical Research
Performing Camden, New Jersey 08103
Organization:

Contract Number: N01-CB-23868
Starting Date: June 1, 1972 Expiration Date: Nov. 30, 1975

Goal: (1) To develop techniques for the reliable isolation of human breast cancer cells from solid tumors and to stimulate their replication in tissue culture; (2) To supply these cells for use in immunodiagnostic assays.

Approach: Tumor cells are obtained from fresh biopsy specimens selected by a responsible pathologist. The primary cells are seeded in various culture media and supplements previously tested for their ability to support the viability and growth of a standard human breast tumor cell line: BT-20. The neoplastic nature of the isolated cells is determined by Pap's smears, by their morphological appearance in culture, by recognized cytological markers such as nuclear/cytoplasmic ratio and by their fine structure as observed by electron microscopy.

Progress: Techniques for the isolation of breast carcinoma cells have been perfected and are successful with 75% of the specimens received. Cultures of primary tumor epithelium are still viable 20 months after explantation. Cell viability is extended with a medium enriched with extra glucose, amino-acids and vitamins. Mitoses are induced with a culture supernatant conditioned by human embryonic cells, with regenerated liver extracts and with cGMP. Nutritional factors appear to be supplied by fetal calf serum absorbed with activated charcoal and reconstituted by addition of hydrocortisone.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Dr. Chou-Chik Ting
Program: Breast Cancer Diagnosis Site Visit Date: 2/28/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $33,000

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography - A Comparative Study

Principal Investigator: Richard Lester, M.D.
Name/ Address Duke University
Performing Durham, North Carolina 27710
Organization:

Contract Number: N01-CB-44011
Starting Date: June 28, 1974 Expiration Date: March 27, 1976

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: This project will be developed among three institutions, University of Texas M.D. Anderson Hospital and Tumor Institute, Duke University Medical Center, Stella and Charles Guttman Breast Diagnostic Institute. It is proposed to assess the mammographic image formation and information-transmitting characteristics of each process. Such factors as radiographic contrast, latitude, sensitivity, and modulation transfer function affect the ultimate transmittal of information. Methods to assess these factors will be used to determine optimum techniques in both types of radiography. Specifically, the Duke staff will provide spectrometric services such that the two modalities can be compared under known spectral conditions. Preliminary measurements will be carried out at the Image Research Center at the University of Chicago under subcontract; these will include modulation transfer function for the baseline film mammographic recording medium and the screen film mammographic system manufactured by DuPont. An advisory and monitoring body will be constituted which will render judgment upon the technical quality of work performed by each of the participating institutions. Since the subjective evaluation of the individual examinations should not depend entirely upon the principal investigators as individual bias will intrude, the cephalocaudal films from all institutions will be reviewed by a panel, the members of which will be named by the Diagnostic Radiology Committee of the National Cancer Institute.

Progress: The Duke University clinical group has prepared and is now activating detailed protocols for patient radiographic evaluation. The physics group has set up an optical bench with pinhole collimation to reduce photon flux and with a high-speed mechanical chopper to select photons excited by different segments of the tube waveform. Arrangements have been completed with the Anderson and Guttman groups for machine evaluation (kVp, etc.) and patient dose measurements by uniform methods.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/27/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $44,972 (nine months)

CONTRACT RESEARCH SUMMARY

Title: Purification of an Antigen from Human Mammary Carcinoma

Principal Investigator: Donald M. Marcus, M.D.
Name/Address Albert Einstein College of Medicine
Performing Bronx, New York 10461
Organization:

Contract Number: N01-CB-23871
Starting Date: June 20, 1972 Expiration Date: March 19, 1976

Goal: (1) To identify antigens associated with breast cancer and to develop a quantitative radioimmunoassay (RIA) for use in immunodiagnosis of breast cancer; (2) to study the correlation between *in vitro* assays for cell mediated immunity to breast tumors and the clinical status and course of patients, and to identify antigens that elicit cellular immunity.

Approach: (1) To develop antisera specific for tumor-associated antigens, purify the antigens by immunoadsorbent and biochemical techniques and to use the purified antigens in a double-antibody RIA. (2) To make extracts of primary breast tumors obtained at the time of surgical resection and to test autologous and allogeneic extracts against the lymphocytes of the patient by three assays: (a) a viral plaque assay developed by Dr. B. Bloom; (b) inhibition of leucocyte migration; and (c) incorporation of ^{3}H-thymidine.

Progress: (1) An antigen detected in breast tumors was purified and identified as ferritin. A double-antibody RIA for ferritin in human serum has been developed, and it has been found that serum ferritin levels were elevated preoperatively in 33% (7/21) of patients undergoing surgery for a malignant lesion, and in 75% (63/84) patients with recurrent or metastatic breast tumors. We have also detected acidic isoferritins that appear to have antigenic determinants that differ from normal adult liver and spleen ferritins. (2) We have set up the three *in vitro* assays and have standardized them by employing antigens such as tuberculin and streptokinase-streptodornase that elicit positive skin tests in these patients. Soluble extracts are being prepared from tumors by extraction with 3 M KCl and these extracts are being tested in the *in vitro* systems.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: K. Robert McIntire, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1/18/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $63,750 (nine months)

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Tissue Supply

Principal Investigator: Name/Address Performing Organization:	Paul O'Brien, M.D. Medical University of South Carolina Charleston, South Carolina 29401

Contract Number: N01-CB-23867
Starting Date: June 30, 1972 Expiration Date: June 28, 1976
Goal: To obtain tissue and blood specimens from patients with breast cancer, for use in immunodiagnostic assays.

Approach: At the time of surgery, obtain tumor and normal breast tissue. Obtain serum and peripheral blood lymphocytes before surgical therapy and at regular intervals and thereafter. These specimens are shipped promptly to other investigators in the program for use in immunodiagnostic assays.

Progress: The contract originated in 1972, and since that time more than the minimum number of blood and tissue specimens have been obtained and shipped every year. During the period from January 1974 to March 1, 1975, there were approximately 148 pre-operative blood specimens from patients with malignant tumors, 109 malignant breast tumors, 19 benign breast tumors, and 130 follow-up blood specimens on patients with malignant tumors. Shipments are made on a weekly and monthly basis. In addition to these, there were 13 bloods sent for special studies on patients with breast cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4C)

Project Officer: Ronald Herberman, M.D.
Program: Breast Cancer Diagnosis Site Visit Date; 5/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $80,000

CONTRACT RESEARCH SUMMARY

Title: Fibrocystic Disease of the Female Breast and its Relationship to Mammary Carcinoma

Principal Investigator: David L. Page, M.D.
Name/Address Vanderbilt University
Performing Nashville, Tennessee 37232
Organization:

Contract Number: N01-CB-43871
Starting Date: January 15, 1974 Expiration Date: Jan. 14, 1976
Goal: To determine which lesion or which combination of lesions, if any, in chronic cystic mastopathy leads to the development of mammary carcinoma.

Approach: The investigator is assembling, reviewing and classifying slides from breast biopsies of 1000 women performed 15 or more years ago and diagnosed as cystic disease at that time. All slides will be classified as to component parts of cystic disease according to the Foote and Stewart classification system. Patients will be followed until their death or the development of mammary carcinoma. Each patient, each patient's record, and physician or family will be contacted to secure the desired information. Correlative studies between the component types of cystic disease (including papillary and lobular proliferative lesions) and the development of mammary carcinoma will be undertaken. An attempt will be made to demonstrate how various kinds of cystic disease lesions are associated with the development of mammary carcinoma.

Progress: 1007 individuals with benign biopsies were accepted for review and categorized by type of cystic disease, following completion of pilot study which tested feasibility of follow-up contact and questionnaire content. 826 letters of inquiry were mailed with responses received from 461 subjects. Of the 461 subjects who have responded, questionnaires have been completed for 417 subjects. At present, 365 initial letters have had no response or have been returned because of an incorrect address.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $57,300

CONTRACT RESEARCH SUMMARY

Title: Analysis of Breast Secretions

Principal Investigator: Name/Address Performing Organization:	Nicholas L. Petrakis, M.D. University of California San Francisco, California 94143

Contract Number: N01-CB-33882
Starting Date: May 15, 1973 Expiration Date: May 13, 1976
Goal: To evaluate Sartorius Breast pump technique as a means of breast cancer detection, using cytological, biochemical and virological analysis of secreted material.

Approach: Several studies are being pursued: (a) correlation of cytologic findings on nipple aspirates in women with undiagnosed breast masses, and comparison with mammography and surgical pathology. Attempts are being made to find tumor cells, cellular atypia, and/or specific biochemical changes, (b) aspirations from women attending the ACS-NCI Breast Cancer Demonstration Center at Merritt Hospital in Oakland.

Progress: Nipple aspirations have been conducted on about 2600 women in the San Francisco Bay area and on 1000 women in Santa Barbara. As of December 1974, over 150 women with suspected breast lesions were subjected to nipple aspiration and correlated with mammography and pathology. Since September 1974, fluid was aspirated from 610 women at the NCI-ACS Breast Cancer Detection Center at Merritt Hospital. It is expected that by May 1975 we will have studied 1000 women and will begin to evaluate cytology in conjunction with mammography, thermography and physical examination. In a partial analysis of fluids from 818 women, 39 (4.7%) had "atypical" cells. These were subdivided as follows: atypicality in normal women, 17/735 (2.3%); benign disease 2/19 (10%); carcinomas 5/10 (50%); previous mastectomy 15/54 (29%). These findings suggest that atypicality of duct cells may be a marker in breast cancer. In Santa Barbara, Dr. Sartorius has found 18 previously undetectable carcinomas by duct catheterization and contrast mammography.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/25/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $170,000

CONTRACT RESEARCH SUMMARY

Title: High Resolution Electronic Mammography for Early Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Donald Sashin, Ph.D. University of Pittsburgh Pittsburgh, Pennsylvania 15260

Contract Number: N01-CB-33904
Starting Date: June 20, 1973 Expiration Date: Sept. 30, 1975

Goal: Develop electronic mammographic technique designed for mass screening for early breast cancer detection. This system has the advantages of rapid low cost operation, small film format, reduced radiation exposure to the patient, selective frequency enhancement, low noise and reduced film storage costs.

Approach: Electronic mammography involves substitution of image intensification and TV techniques for x-ray film, increasing the efficiency with which x-rays can be recorded. The program consists of two parts: (1) design and construction of a prototype system optimized for visualization of soft tissues and (2) evaluation of the system regarding its ability to display clinical details relative to present mammographic systems. In this method a mammographic image display on a intensifying screen is viewed by a high resolution TV camera. The TV image is displayed on a cathode ray tube and photographed automatically by a 70 mm roll film camera.

Progress: The system has been designed and evaluated with phantoms and in the clinical setting with regard to resolution, noise and clinical performance. Each component of the system has been carefully optimized to insure the best final image quality. In the phantom studies the system exceeded 70mm photofluorographic mammography in resolution while requiring comparable radiation exposure. In the preliminary clinical study, small tumors and other pathology were demonstrated. Modifications have been made to the original system design to further improve performance. These include a moving slit technique and redesign of some of the electronic circuits. Phantom measurements of the system performance with these new improvements show that the radiation dose is reduced and the image noise is lowered while the image contrast is improved by about 100% compared to the earlier performance of this system. An advanced system has been built and is being evaluated and optimized in the clinical setting. Initial clinical images show good detail with a radiation exposure of about 80mR to the skin (about 1/3 of the exposure that was required for the dose mammography).

Significance for Cancer Research (NCP Objective 5 Approach 3)
Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 8/27/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $28,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Analysis of Human Breast Cyst Fluid

Principal Investigator: Name/Address Performing Organization:	Morton K. Schwartz, Ph.D. Sloan-Kettering Institute for Cancer Research 410 East 68th Street New York, New York 10021

Contract Number:
Starting Date: Expiration Date:

Goal: To search for a diagnostic breast cancer marker in human breast cyst fluid using biochemical and immunochemical techniques.

Approach: 150 breast cyst fluid specimens will be analyzed for cortisol, the androgens, estrogens, progesterone, FSH, LH and prolactin. In addition, tumor associated antigens, minerals, proteins and nitrogenous constituents, DNA and polyamines, lipids, and enzymes will be studied. The objectives will be to determine if the biochemical analysis of human breast cyst fluid can predict those patients who already have or will subsequently develop breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 12/11/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $100,822

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography - A Comparative Study

Principal Investigator: Philip Strax, M.D.
Name/Address Performing Organization: Guttman Institute
New York, New York 10028

Contract Number: NO1-CB-44012
Starting Date: June 28, 1974 Expiration Date: March 27, 1976

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: This project is being carried out among three institutions, University of Texas, M.D. Anderson Hospital and Tumor Institute, Duke University Medical Center, Stella and Charles Guttman Breast Diagnostic Institute. Mammographic image-formation and information-transmitting characteristics of each process will be assessed by way of radiographic contrast, latitude, sensitivity and modulation transfer function. Preliminary measurements will be carried out at the Image Research Center at the University of Chicago under subcontract. An advisory and monitoring body set up by the Diagnostic Radiology Committee of NCI will review the technical quality of work performed by the participating institutions.

Progress: Guttman Institute is using Kodak (AA) films with the Senograph and moly filter in both positions plus cranio-caudad projections on LD films with Senograph and moly filter and XR plates with Senograph and .5mm. Al filter on each woman. One hundred twenty-six such sets have been produced to date. Radiation outputs at M.D. Anderson Hospital have been obtained (air measurements: R/100 mAs). A Victoreen 415 A intercomparison chamber was used with an electrometer and digital read-out. 415A was re-calibrated at Anderson in terms of R/Coul. Measurements were taken for tungsten tube (no added filtration), and a moly tube (0.03 mm Mo. added) for the AA, Low Dose and xeroradiographic techniques. Thermoluminescent dosimetry in the Low energy range for 0.1 to 25 R. is in progress, so that skin entrance dose measurements may be accomplished.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/24/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $26,175 (nine months)

CONTRACT RESEARCH SUMMARY

Title: Steroid Excretion Study in Patients with Breast Cancer

Principal Investigator: Per Vestergaard, M.D.
Name/Address Performing Organization: Research Foundation for Mental Hygiene
Orangeburg, New York 10962

Contract Number: N01-CB-71465
Starting Date: June 28, 1967 Expiration Date: Dec. 26, 1975
Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: The analysis of steroid hormones is based on column chromatography, the column consisting of fine, capillary teflon tubings kept in a constant temperature bath. After the urine extracts are deposited at the head of the column, automation takes over so that all steps become highly reproducible. Even though the analysis per se is nonspecific, the high number of fractions collected allows for fine separation of metabolites without cross-contamination.

Progress: The special instrumentation developed under the contract has been described in a series of papers accepted for publication. A monograph describing the steroid methodology is in preparation. Data from the steroid hormone studies in patients and controls are being evaluated. Final evaluation for publication is now being undertaken.

Significance for Cancer Research (NCP Objective 6 Approach 6)

Project Officer: Ihor Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/28/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: John D. Wallace
Name/Address Thomas Jefferson University
Preforming Philadelphia, Pennsylvania 19107
Organization:

Contract Number: N01-CN-35027
Starting Date: June 28, 1973 Expiration Date: June 27, 1975

Goal: To evaluate the contribution thermography may make in increasing the effectiveness and/or efficiency of breast cancer screening programs.

Approach: 20,000 women will be screened by three methods: clinical examination, thermography and xerography, each modality being evaluated independently. To obtain the study population, women aged 45-65 from a variety of sources (American Cancer Society screenees, Bell Telephone Company employees, etc.) will be invited to participate. All findings will be reviewed by medical coordinator who will be responsible for follow-up medical recommendations. This project is being run in conjunction with the Health Insurance Plan of Greater New York, N01-CN-35021.

Progress: The screening program started during the last quarter of 1973. As of the end of 1974, 10,478 women had had an initial examination. Of these, 379 had significant finding and diagnostic workup including biopsy or aspiration where recommended. Of this number, there were 62 with a positive diagnosis of malignant neoplasm, 22 with a diagnosis still pending, 52 where the attending physician has deferred diagnostic workup, 226 with negative diagnostic findings, and in 17 cases the patient has refused to have a biopsy. Of those diagnosed with breast cancer, 32 percent had a positive thermogram, while mammography was positive in 77 percent of the cases and there were clinical findings in 61 percent.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Harvey Geller
Program: Cancer Control Site Visit Date: March 24-25, 1975
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $1,121,352

CONTRACT RESEARCH SUMMARY

Title: Determinations of Steroid Sulfate Content in Blood to be Used as Diagnostic Parameters in Breast Cancer

Principal Investigator: Federico Welsch, M.D., Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology, Inc.
Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-43898
Starting Date: January 1, 1974 Expiration Date: Dec. 31, 1975
Goal: To analyze blood steroid sulfate levels to serve as possible diagnostic and/or prognostic parameters in breast cancer.

Approach: Estrone sulfate, dehydroepiandrosterone sulfate and androsterone sulfate will be measured in the plasma of at least 100 patients per group, either with diagnosed breast cancer or diagnosed benign breast tumors or apparently normal controls using radioimmunoassay techniques. These measurements can be used to indicate possible changes in adrenal steroid production in postmenopausal women and adrenal and ovarian activity in young women occurring after the development of breast cancer. It would be an attempt to find a unique metabolite in blood which might be characteristic of breast cancer.

Progress: Values for estrone sulfate, dehydroepiandrosterone sulfate, and androsterone sulfate have been determined in a normal population. Sixty-one blood samples have been received from Dr. Horning at Baylor and have been analyzed. Fifteen samples received from Dr. Lynch at Creighton University have been analyzed. All results are being sent to the Project Officer at NCI.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 9/4/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $98,300

CONTRACT RESEARCH SUMMARY

Title: Automated Methods for Determination of Steroid Metabolites

Principal Investigator: Federico Welsch, M.D., Ph.D.
Name/Address Performing Organization: The Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-60968
Starting Date: June 10, 1966 Expiration Date: June 8, 1976

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: This analysis of steroid hormones is based on paper chromatography with uniquely designed triple wavelength readout permitting an automated Allen correction to be incorporated. The methodology is highly specific and reproducible, permitting the analysis of a spectrum of hormonal metabolites on 5-10% aliquot of a 24-hour specimen.

Progress: During the year 1973-1974, the contractor exceeded the specifications for work load by 200% without additional cost. The laboratory serves as the analytical arm of the clinical study designed to develop a predictive index for spotting early recurrences after radical mastectomy. When patient accrual and follow-up ends, the contractor will be checking out one year postmastectomy values of urinary steroids. During the present fiscal year 740 urine samples were analysed, about 250 for Breast Cancer Task Force Study 8000, and about 490 for the NCI intramural program (Dr. Tormey).

Significance for Cancer Research (NCP Objective 6 Approach 6)

Project Officer, Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1971
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator: David E. Anderson, Ph.D.
Name/Address Performing Organization: The University of Texas System Cancer Center
Houston, Texas 77025

Contract Number: NO1-CB-43939
Starting Date: June 17, 1974 Expiration Date: September 15, 1975

Goal: To measure aryl hydrocarbon hydroxylase (AHH) inducibility levels in cultured lymphocytes from members of high-and low-risk breast cancer families for possible use as a genetic marker.

Approach: Lymphocytes are isolated from fresh human blood specimens using Ficoll-Hypaque density gradient centrifugation. The cells are cultured in a medium of Eagle's medium and fetal calf serum, with stimulation of mitosis by a mixture of phytohemmaglutinin and pokeweed mitogen. At the end of three days, experimental tubes are induced with a solution of 3-methylcholanthrene in acetone. Control tubes receive an equal volume of acetone. At the end of another 24 hours, the cells are harvested and assayed for AHH activity by a spectrophotofluorometric method using benzypyrene as substrate. Inducibility is expressed as the ratio of the activity of induced cells over the activity of control cells.

Progress: Primary effort has been directed toward establishing and standardizing the lymphocyte culture and AHH assay protocol. Attempts are now being made to increase the reproducibility by further modification of the procedures. Inducibility data have been obtained on 11 premenopausal and 18 postmenopausal patients, and 37 unaffected women. The patients and controls show wide individual variability. However, repeat cultures from the same subjects are also variable. Consequently, increased reproducibility of the assay will be essential to provide meaningful inducibility data. Blood samples are now being collected from patients, their unaffected sisters, and controls, and the lymphocytes are being frozen to be processed when the assay is more reproducible.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Daniel Nebert, M.D. and Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/30/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 75 Funds: none

CONTRACT RESEARCH SUMMARY

Title: Investigation of Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: David E. Anderson, Ph.D.
Name/Address Performing Organization: University of Texas System Cancer Center
Houston, Texas 77025

Contract Number: N01-CB-44004
Starting Date: June 16, 1974 Expiration Date: March 15, 1976
Goal: To investigate a series of polymorphic genetic markers in high and low risk breast cancer families which might assist in the definition of high-risk individuals.

Approach: The ABO, Rh, MNS, P, K, Fy^a and Jk^a blood group types; serum haptoglobin types; alkaline phosphatase and phosphoglucomutase red blood cell types; and chromosome abnormalities and/or variants will be determined from blood specimens. Comparisons will be made between breast cancer patients from high-risk families and normal control women matched for menopausal status, age and race. Another comparison will be between the unaffected sisters of patients from high-risk families and matched controls. For the chromosomal studies selected premenopausal and postmenopausal patients and their unaffected sisters from high-risk families will be compared with matched controls.

Progress: Blood specimens have been collected from approximately 70 patients, unaffected sisters, and controls, and processed on a routine basis. The numbers are as yet too small to provide meaningful results. Chromosome analyses have been completed on 10 selected high-risk patients and 5 of their unaffected sisters. Dicentric and marker chromosomes were highly frequent in each patient that had previously undergone radiation therapy. These changes were also detected in one cell of one of the two untreated patients and in 3 of 5 of the unaffected sisters. Effort is now being made to select individuals for chromosome studies who have had no prior radiation therapy.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Joseph Fraumeni, M.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/30/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $49,494 (nine months)

CONTRACT RESEARCH SUMMARY

Title: Investigations of Histocompatibility Antigens and Other Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: Frans J. Cleton, M.D.
Name/Address Performing Organization: The Netherlands Cancer Institute
Amsterdam, The Netherlands

Contract Number: N01-CB-43940
Starting Date: June 18, 1974 Expiration Date: June 16, 1976

Goal: To study linkage of certain genetic markers with breast cancer in an attempt to select high risk groups in the population for the development of breast cancer.

Approach: HL-A haplotypes will be studied in families with a high incidence of breast cancer. A large number of other genetic markers will also be investigated, such as blood groups, immunoglobulins, red cell isoenzymes and chromosomal polymorphisms. As a peripheral project, group specific antigens will be studied, using a sensitive micro-immune diffusion technique with anti-mouse MTV sera and milk of lactating family members will be collected for study of a possible human MTV using electron microscopy and biochemical techniques. Plasma prolactin levels will be measured in all high risk family members. Because there is some indication that the histology of familial breast cancer is different with respect to lymphocytic infiltration from general breast cancer, a histological classification of all tumor biopsies will be carried out.

Progress: Up to February 1975, HL-A antigens were typed in all available members of three generations in 9 high-risk families. All other genetic markers and chromosomal polymorphisms were studied in 50 selected relatives of breast cancer patients in 4 of these families. It is too early to evaluate the results of these studies. Preliminary results of the studies on histology show that there is a high frequency of medullary carcinoma and a marked lymphocytic infiltration in the majority of tumors investigated.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $50,130

CONTRACT RESEARCH SUMMARY

Title: An International Study on the Role of Overweight in Breast Cancer Epidemiology

Principal Investigator: Frits de Waard, M.D.
Name/Address Queen Wilhelmina Fund
Performing Amsterdam, THE NETHERLANDS
Organization:

Contract Number: N01-CB-33885
Starting Date: July 1, 1973 Expiration Date: June 26, 1976

Goal: To produce age-specific incidence curves of breast cancer for various subgroups differing in body weight and height within geographically defined populations to determine if anthropometric measurements have an effect on the level and shape of the breast cancer incidence curves.

Approach: Calculate incidence rates on the basis of case-control comparisons in regions where a population-based cancer registry exists.

Progress: Data have been collected on 1080 breast cancer cases and 5000 population controls in the cities of Rotterdam and the Hague and the province of Friesland. Work on the controls in the Hague and Friesland has been completed. Preliminary results were presented at NCI on September 30. Both weight and height have a profound effect on the level and shape of the breast cancer incidence curve. Data on age, parity, age at first birth, body weight and height at first diagnosis in all new breast cancer cases occurring over a one year period and a representative sampling of controls in the city of Utrecht and its suburbs will be collected. Age-specific incidence curves of breast cancer for various groups of Dutch women varying in body weight and height and age at first birth will be calculated.

Significance for Cancer Research(NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/4/73
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $11,500

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator: Jack Fishman, Ph.D.
Name/Address Performing Organization:
Montefiore Hospital and Medical Center
Bronx, New York 10467

Contract Number: N01-CB-44002
Starting Date: June 24, 1974 Expiration Date: June 22, 1976

Goal: To evaluate the possible role of genetic factors in the expression of specific biochemical parameters in breast cancer pathophysiology.

Approach: Endocrine assays will be done on blood and urine of premenopausal females from families with a high incidence of breast cancer and from matched control families. Morning blood samples obtained every second day throughout the menstrual cycle will be assayed for FSH,LH,E_3,E_2,E_1, progesterone and prolactin. Nocturnal urines (7 P.M. to 7 A.M.) will be assayed for 11 to 13 estrogenic, androgenic and corticosteroid metabolites. An attempt will be made to correlate familial susceptibility to breast cancer with endocrine parameters.

Progress: Plasma and urine samples from 14 women have been received. Endocrine assays of plasma hormones in 12 subjects are essentially complete. Due to extremely low concentration of plasma E_3 found in 4 subjects, this measurement has not been made in the other subjects. Steroid assays on daily nocturnal urines are essentially complete in 7 subjects. The androgens are being measured separately as sulfates and glucuronides. The glucuronides of estrogens have been measured in the 7 subjects and the sulfates in 2 subjects. 2-Methoxyestrone assays are still pending.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice Radovich, Ph.D., Charles Brown, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/29/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $192,000

CONTRACT RESEARCH SUMMARY

Title: Investigations of Possible Correlations Between Morphological and Epidemiological Characteristics of Breast Cancer

Principal Investigator:	H. Stephen Gallager, M.D.
Name/Address	The University System Cancer Center
Performing	M.D. Anderson Hospital and Tumor Institute
Organization:	Houston, Texas 77025

Contract Number:
Starting Date: Expiration Date:

Goal: To demonstrate morphologic markers characteristic of familial types of breast disease in order to identify selected high risk populations.

Approach: A previously developed system for the profiling of the morphologic characteristics of breast cancer which integrates information from gross and histologic examination, mammography, and specimen radiography and requires the rough quantitative evaluation of some 39 factors in these three groups will be validated and refined. The breast cancer specimens will be derived from three major risk groups which have been characterized genetically. These consist of 114 patients with mothers with cancer pedigrees, 97 patients with sisters with cancer pedigrees and 111 patients with second order pedigrees for breast cancer. The findings will be compared with 300 patients without family histories.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $36,247

CONTRACT RESEARCH SUMMARY

Title: Combined Study on the Possible Association of Dietary Factors and Non-Contraceptive Estrogens with Breast Cancer

Principal Investigator: Tomio Hirohata, M.D.
Name/Address University of Hawaii at Manoa
Performing Cancer Center of Hawaii
Organization: Honolulu, Hawaii 96822

Contract Number:
Starting Date: Expiration Date:

Goal: To determine if there is an association of breast cancer occurrence with the use of non-contraceptive estrogens and with specific dietary factors in postmenopausal women.

Approach: A case-control (retrospective) design is planned in which a validated questionnaire will be employed for information on past history of drug usage (including exogenous estrogens) and usual dietary intake. Breast cancer cases from three selected populations (ages 45-74) will be used: (1) the Caucasians in Hawaii who are at high risk for breast cancer; (2) the Japanese in Hawaii who are at intermediate risk; and (3) the Japanese in Fukuoka, Japan representing a low risk population. Two controls, matched by race and age will be used for each case.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $97,267

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer and Estriol Dynamics

Principal Investigator: Christopher Longcope, M.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-33902
Starting Date: June 1, 1973 Expiration Date: May 30, 1976

Goal: To determine the production and metabolic clearance of estriol in relation to high and low excretors in order to determine the physiological basis for repeated differences between high and low breast cancer-prone individuals.

Approach: In this research the investigator plans to apply proven techniques to determine the sources and blood production rates of estriol in (1) normal women with high and low urinary E_3/E_2+E_1 ratios; the women to range in age from 21 to 90 years of age and to be nulliparous as well as parous; and (2) women with breast cancer. The aim is to determine the pattern of precursor-estriol relationship and blood production rates in normal women who have high or low urinary E_3/E_2+E_1 ratios, and in women with breast cancer and to correlate the dynamics as determined by measurements made from the free estriol pool in blood with those measurements made from estrogen metabolites in urine. These data will be used to delineate better the normal dynamics of estriol production and metabolism and the role of estriol in human breast cancer.

Progress: Studies have been carried out on 24 subjects with infusions of androstenedione, estrone, estradiol and estriol in the follicular and luteal phases of the cycle. The metabolism of estriol is similar in both phases of the cycle, but the production rate of estriol is slightly higher in the luteal as compared to the follicular phase. Women with high ratios of E_3/E_2+E_1 in their urines have similar production and metabolic clearance rates to women whose ratio is low. Androstenedione is an important source of estriol in both groups but in women with high ratios it contributes more to estriol than in the low ratio group.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer. Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/23/73
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $42,450

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Family Resources

Principal Investigator: Henry T. Lynch, M.D.
Name/Address Creighton University
Performing School of Medicine
Organization: Omaha, Nebraska 68178

Contract Number: N01-CB-33901
Starting Date: June 30, 1973 Expiration Date: June 28, 1976
Goal: To establish a research pool of about 80 well-documented breast cancer-prone families to be used for medical genetic studies.

Approach: There is continuous updating of genealogy and tumor history by personal interviews with patients and physicians and by telephone calls and letters. A critical analysis of these families is continued in a search for basic genetic mechanisms through the study of genealogy and varying combinations of malignant neoplasms as well as other medical disorders in this series of breast cancer-prone families. Pedigrees with detailed descriptions which are compiled on each family are available for review by each contractor who expresses an interest in studying these families. Access to the resource by qualified investigators (with separate funding) is subjected to approval by the contractor in consultation with the Breast Cancer Task Force.

Progress: Twenty-four new families have been ascertained. The resource is currently supplying biological specimens for: 1) J. E. Fishman, Ph.D., Montefiore Hospital for hormonal studies on urine and blood plasma collected during the entire menstrual cycle of high and low risk women (12/30 high risk women and 4/30 low risk women have been completed); 2) P.I. Terasaki, Ph.D., UCLA and N. Petrakis, M.D., UCSF, for HL-A and genetic markers in breast cancer families (ten breast cancer families have been sampled; 199 blood specimens have been shipped); G. Mulcahy, M.D., Jersey City Medical Center, is reviewing pathology reports and available slides on these families and others from the resource; 3) steroid sulphate analysis in urine and plasma by Baylor College of Medicine and the Worcester Foundation on samples from 20 post-menopausal high risk women who have not had breast cancer and two controls; 4) other investigators (CEA, SV-40 virus testing of skin fibroblasts, and screening for bovine leukemia virus, Epstein Barr Virus, and feline leukemia virus).

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/10/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $61,415

CONTRACT RESEARCH SUMMARY

Title: Investigations of Histocompatibility Antigens and Other Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: Henry T. Lynch, M.D.
Name/Address Performing Organization: Creighton University
School of Medicine
Omaha, Nebraska 68178

Contract Number: N01-CB-44003
Starting Date: June 17, 1974 Expiration Date: June 15, 1976

Goal: To investigate histocompatibility antigens and other genetic polymorphisms in high and low risk breast cancer families.

Approach: HL-A markers, genetic polymorphic markers such as red blood cell enzymes, (acid phosphatase, adenosine deaminase, etc.) serum proteins (haptoglobins a-chain, group-specific component, etc.), and blood group systems (ABO, Duffy, Lewis, MN, etc.) and specific pathologic diagnoses (all histologic varieties of cancer retrospectively in the family context) are being evaluated in approximately 20 multiple cancer case families selected from the total clinical pool of 80 high risk families and kindreds.

Progress: Ten of twenty multiple case families have been sampled (3 to 73 individuals per family) since the contract was initiated. The families included 2 families with site specific breast cancer, 2 with predominance of breast, sarcoma and brain tumors, 2 with breast and ovary, and 4 with breast and colon cancers. A total of 199 individuals have been seen and blood samples were obtained. Specimens have been sent to the investigators, Dr. Terasaki, Univ. of California, Los Angeles, for HL-A typing, and Dr. Petrakis, University of California, San Francisco, for genetic polymorphism studies. Analysis has been completed on all individuals for the following systems: ABO, Duffy A and B, Lewis A and B, MNS, RH (Cc, Dd, Ee), group-specific component, and haptoglobin (a-chain). Analysis for acid phosphatase, adenylate kinase, esterase D, and phosphoglucomutase-1 are in progress. Preliminary technical work is in progress on the remaining systems: adenosine deaminase, soluble glutamate-pyruvate transaminase, peptidase A, A1-antitrypsin, and the third component of complement. The majority of pathology slides and reports, which represent the varieties of cancers diagnosed in all of the breast cancer families, have been forwarded to Dr. Mulcahy, Jersey City Med. Center, for his evaluation on a double blind basis.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/10/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $99,975

CONTRACT RESEARCH SUMMARY

Title: Investigation of Possible Correlations Between Dietary Factors and Epidemiological Characteristics of Breast Cancer

Principal Investigator: Baruch Modan, M.D.
Name/Address Chaim Sheba Medical Center, Tel Hashomer and Tel Aviv
Performing University Medical School
Organization: Tel Hashomer, Israel

Contract Number:
Starting Date: Expiration Date:

Goal: To carry out epidemiological investigations of selected population groups to elucidate the possible role of diet and/or nutritional status on the incidence, pathogenesis and natural history of breast cancer.

Approach: A case-control study using a validated questionnaire will be conducted to determine whether dietary components particularly fats and (low) cellulose, may serve as risk factors in the etiology of breast cancer. The study population will be composed of 500 newly diagnosed breast cancer cases of which 300 will be European and American born and 200 will be Asian-African born. The 1000 controls will be matched for age, parity, menopausal status, menarche, country of origin, period of immigration to Israel, etc. The women will also be characterized with respect to anthropometric measurements.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $41,247

CONTRACT RESEARCH SUMMARY

Title: Investigations on the Effect of Exogenous Estrogens on the Occurrence and Natural History of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Robert W. Morgan, M.D. University of Toronto Toronto, Ontario, Canada

Contract Number:

Starting Date: Expiration Date:

Goal: To examine the risk of breast cancer, benign breast disease, and endometrial cancer in menopausal and postmenopausal women who had received estrogen therapy for other than contraceptive purposes.

Approach: A retrospective cohort study design will be used. The study group (Cohort A) will be composed of 1200 women (ages 40-55) in Toronto who between 1960 and 1970 were started on long-term estrogen therapy for menopausal complaints. All drug use and epidemiologic data will be gathered from hospital records, Ontario Cancer Registry, Ontario Cancer Foundation, private physician records and phone and home interviews. The incidence rates for breast cancer, benign breast disease and endometrial cancer will be compared with those of the general population of Ontario (Cohort B) for whom age-specific rates on cancer incidence and mortality are now available. The incidence rates for the study population will also be compared with another 1200 matched control (Cohort C, unexposed to estrogens) group. An estimation will be made of relative and attributable risk of developing benign or malignant breast disease or endometrial carcinoma and of dose-response relationships of estrogen to disease.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.

Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY'75 Funds: $74,880

CONTRACT RESEARCH SUMMARY

Title: A Study on Familiality of Breast Cancer in Iceland

Principal Investigators: Hrafn Tulinius, M.D., Prof. O. Bjarnason
Name/Address International Agency for Research
Performing on Cancer
Organization: Lyon, FRANCE

Contract Number: N01-CB-43973
Starting Date: June 30, 1973 Expiration Date: June 28, 1976

Goal: To determine whether there are hereditary characteristics which might be of use in the identification of women having a high risk of developing breast cancer.

Approach: The unique facilities available in Iceland for the study will be utilized. These include (1) a nationwide cancer registry functioning since 1954 (about 800 cases), (2) a computer-based National Roster since 1952, and (3) a family linkage file linking every individual alive after 1910 to his parents and children. If an excess risk is found, an effort will be made to (1) correlate familial risk with recognized epidemiological risk factors for breast cancer, (2) quantify the risk attached to different types of relationships, and (3) for each class of relative, examine the association, if any, between the degree of risk and the age of the proband case and age of appearance of cancer among these relatives.

Progress: Family linkage file is under construction and is at different levels of completion. It can be considered 100% complete for those born in or after 1916, and can identify all persons alive at the time of 1910 census. A birth record from 1840 is being reconstructed and is complete for about half the population. Analysis of incidence data from 1911-1972 has shown a marked increase in breast cancer during the period and a corresponding change in the shape of the age incidence curve. This change in shape can be explained solely as a cohort effect. Preliminary results are also available on expected and observed rates of breast cancer in defined relatives of the 239 cases of breast cancer who were born in 1916 or later.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $35,000

CONTRACT RESEARCH SUMMARY

Title: Investigation of Possible Correlations Between Morphological and Epidemiological Characteristics of Breast Cancer

Principal Investigator: Bjorn Stenkvist, M.D.
Name/Address Department of Clinical Cytology
Performing University Hospital, Uppsala
Organization: S-750 14 Uppsala, Sweden

Contract Number:
Starting Date: Expiration Date:

Goal: To ascertain possible correlations between morphologic variables such as cytologic and histopathologic classifications and established epidemiological risk factors of breast cancer, such as menarche, age at first pregnancy, familial history, etc.

Approach: This study will be composed of two phases: Phase I will consist of a pilot study of histopathologic material from 110 breast cancers from four counties in Sweden utilizing the computerized Central Swedish Cancer Registry. The tumors will be classified histologically according to WHO (Scarrf and Torloni 1968) and Ackerman's Classification (1970). Another aim of this project is to make a quantitative evaluation of a number of morphological characteristics by computer analysis of tumor cell populations utilizing a Leitz MPV II scanning photometer connected to a PDP-8 computer with 24k of core, and relate these to the standard classification. Planning for the epidemiologic aspects of the study will also be completed under this phase. All epidemiologic data will be collected on each patient and computerized. Under Phase II statistical analysis of the data will be made with application of the newly established morphologic classifications to the epidemiologic variables.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast CAncer Steering Committee
FY'75 Funds: $92,778

CONTRACT RESEARCH SUMMARY

Title: Growth Alteration of Mammary Neoplastic Cells Obtained by Manipulation of Cellular Environment

Principal Investigator: Creed W. Abell, Ph.D.
Name/Address University of Texas Medical Branch
Performing Galveston, Texas 77550
Organization:

Contract Number: N01 CB-43906
Starting Date : June 10, 1974 Expiration Date: June 9, 1976
Goal: To manipulate cellular environment *in vivo* and *in vitro* in order to limit preferentially the growth of neoplastic cells of mammary origin.

Approach: To evaluate the effects of phenylalanine ammonia lyase (PAL) and phenylalanine restriction on mammary tumors maintained *in vitro*, and also to evaluate both on mammary tumors of mice and rats as well as on human breast tumors growing in nude-athymic mice. PAL from *Sporobolomyces pararoseus* which is selective for phenylalanine will be purified and its effects will be evaluated on mammary tumors both *in vivo* and *in vitro*.

Progress: Approximately 2 kg of *Rhodotorula glutinis* are produced per week for the production of PAL with an activity of 2 to 2.5 units 3/gms yeast. Purification procedures for PAL have been improved to the point that a 23% recovery of PAL can be obtained in 7 days *versus* the previous 10% in 14 days. The therapeutic effect of PAL (300 units/kg for 5 days) and diets restricted to low levels of phenylalanine have been evaluated individually against the BW10232 mammary tumor of the C57 black mouse. PAL inhibited tumor growth during the 5 day period only. The restricted diets inhibited tumor growth but also caused appreciable weight loss of the host. In studies of amino acid blood levels in C57 black mice and a squirrel monkey given PAL it was found not only to deaminate phenylalanine and tyrosine but also to reduce other levels of amino acids in the plasma. Enzyme clearance studies of PAL in the plasma of C57 black mice were studied, the results to date suggest that PAL is cleared rapidly upon repeated administration. Other studies initiated in this contract period were: levels of amino acids in the BW10232 tumor; the effects of PAL on the human tumor cell line HBT-3 *in vitro*; and, the production and purification of PAL from *Ustilago bordei* and from *Sporobolomyces pararoseus*.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/14/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $98,000

CONTRACT RESEARCH SUMMARY

Title: Identification of Cell Types in Cultures of Normal and Neoplastic Breast Tissues

Principal Investigator: Samuel Abraham, Ph.D.
Name/Address
Performing
Organization:
Children's Hospital
Oakland, California 94609

Contract Number: N01-CB-33906
Starting Date: June 27, 1973 Expiration Date: June 26, 1976

Goal: To identify cell markers in normal and neoplastic breast cultures of mouse, dog and human origin.

Approach: Antisera to be prepared from: intact cells, cell membranes, microsomes, specific enzymes or cell products. Normal epithelial cells from mouse mammary gland to be separated with methods developed by Abraham, et al. Adipose and fibroblast cells to be obtained from cleared fat pads. Epithelial cell identification will be studied by cell membrane antigens. Specific antibodies produced for each cell type to be tagged with either fluorescent dye or peroxidase. Alternative markers to be explored: anti-myosin for myoepithelial cells; proline hydroxylase and lysyl oxidase for fibroblasts; α-lactalbumin, casein, fatty acid synthesis, for epithelial cells. Other means of identification for cell types, such as hormone binding, to be explored. Unique isoenzymes are a possibility for distinguishing normal and neoplastic cells.

Progress: Mammary gland epithelial cells (MMEC) after enzymatic dissociation from the tissue, found capable in vitro of regenerating those cell surface components responsible for their antigenic properties. Evidence in support of the view that these antigens are not merely produced in vitro, but are present in intact tissue has been obtained. Specific antibodies will distinguish between MMEC and tumor cells (MMTC) are now available. Progress has been made to obtain antibodies specifically directed towards the tumor cells. MMEC and MMTC seem to share many common antigens therefore it appears that intact cell preparations cannot be used to immunize rabbits. From analysis of cell surface components obtained from the two cell types, it appears that different substances useful for antibody production could be obtained. Mild enzymatic treatment of intact cells will solubilize the antigenic materials originally present on the cell surfaces. Analyses of these fractions reveal that tumor cells lack specific components present in normal cells. These materials contain fucose and amino acids capable of iodination by the lactoperoxidase method therefore the contractor concludes they are present on the cell surface and are probably glycoprotein in nature.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Cancer Experimental Biology Site Visit Date: 3/13/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $100,000

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acid Preparations on the Biological Properties of Mammary Carcinomas

Principal Investigator: Aaron Bendich, Ph.D.
Name/Address Performing Organization: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

Contract Number: N01-CB-43904
Starting Date: June 28, 1974 Expiration Date: March 27, 1976

Goal: To determine the relationship between transfer of nucleic acid preparations into mammary cells and possible changes in their growth potential.

Approach: To ascertain: a) the effect of nucleic acids or nucleic acid-containing organelles on the biological properties of normal and malignant cells, especially those of animal and human origin; b) the possible expression of embryonal or other genes after nucleic acid-cell interaction, especially when animal or human sperm is used as the DNA-containing moiety; c) the acquisition of, or change in level of, malignant potential of the target cells, especially those derived from the mammary gland; d) the refinement or development of methods to achieve a, b, and c.

Progress: It has been found that the treatment of "normal" cells with Cytochalasin B leads only to a binucleate condition (i.e., a single endoreduplication), but after transformation with carcinogens or spermatozoa, multinucleates appear or predominate. Human breast tumor HBT-39 and SH-2 cells also show this effect. The technique may prove useful in evaluating oncogenic potential of cells and in following effectiveness of clinical or *in vitro* treatments. Human and animal sperm penetrate the breast tumor and other cells. Changes can be followed in the ultra-violet after acridine orange staining, by the expression of embryonal antigens in previously normal cells, and by determining cultural characteristics.

Significance for Cancer Research (NCP Objective 2,2 Approach 3,3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/22/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $67,500

CONTRACT RESEARCH SUMMARY

Title: Role of the Stroma in the Growth of Neoplastic and Preneoplastic Lesions of the Mammary Gland.

Principal Investigator: Merton R. Bernfield, M.D.
Name/Address Leland Stanford Jr. University
Performing Stanford, California 94305
Organization:

Contract Number: N01-CB-53903
Starting Date: April 15, 1975 Expiration Date: April 14, 1976

Goal: To define the role of the basal lamina, its constituent glycosaminoglycan (GAG), and specific stromal tissue in mammary morphogensis occuring post-natally and during pregnancy.

Approach: Using micro-dissection and enzyme treatments to isolate from mid-pregnant mouse mammary glands pieces of mammary epithelial tissue capable of undergoing in vitro morphogenesis in order to:

1) Establish the presence or absence of GAG within the basal lamina.

2) Elucidate the pattern of basal laminar GAG synthesis, distribution, and turnover during post-natal mammary gland development.

3) Develop procedures for the isolation of post-natal mammary epithelial tissue free of stroma but retaining the basal lamina.

4) Establish whether post-natal mammary epithelial morphogenesis is dependent upon the basal lamina and specific stromal tissue.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/5/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $78,000

CONTRACT RESEARCH SUMMARY

Title: Biological Characterization of Animal Mammary Tumors and Tumor Bank Facility

Principal Investigator: Arthur E. Bogden, Ph.D.
Name/Address Mason Research Institute
Performing Worcester, Massachusetts 01608
Organization:

Contract Number: NO1-CB-31146
Starting Date: June 28, 1963 Expiration Date: June 27, 1976

Goal: To maintain a bank of biologically characterized and monitored (a) in vivo transplantable animal mammary and endocrine related tumors, and (b) human and animal breast tumor cell lines established in in vitro culture, to be used by the BCTF and other selected investigators.

Approach: Freeze-preservation of human breast tumor cell lines, as well as mammary and endocrine related tumors of animal origin: (1) submittal of tumors and cell lines by qualified investigators able to furnish pertinent background information; (2) cell lines tested for viability, plating efficiency, freedom from contamination and tumorigenicity by implanting in nude-athymic mice; (3) biological characterization of transplantable tissues, both pre- and post-freeze-preservation, by determination of growth curves, specific effects on host-organs, host survival, serum hormone levels, histology, karyology, response to ablative procedures; (4) characterized tissue and cell lines preserved in Linde liquid nitrogen freezers according to well recognized, proven procedures.

Progress: Ninety-nine tumors have been shipped to 49 different investigators reflecting 152% and 120% increases respectively during the past year. An increase in number of tumors shipped as well as in those requesting tumors continues. Fifty-three percent of the tumors shipped were of mammary origin and 7% were anterior pituitary tumors characterized by ACTH, prolactin and/or growth hormone secretion. There are 101 different tumors or sublines stored in 2,232 ampules in 6 Linde liquid nitrogen freezers. Twenty-four tumors have been serially transplanted for characterization studies and 13 for replenishing the Tumor Bank inventory. Twelve new tumors were received for characterization and freeze preservation. The Cell Bank received 11 normal and malignant cell lines for characterization and freeze preservation. Cell lines MDA-MB-134, 157, 175, 231 and HBL-100 were designated for complete characterization and freeze preservation into 100 ampules each. Progress continues in preparation of facilities for breeding the nude-athymic mice for testing the tumorigenicity of the cell lines.

Significance for Cancer Research (NCP Objective 3,4,5 Approach______)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/4/71
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $150,000

CONTRACT RESEARCH SUMMARY

Title: Studies on Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Sumner Burstein, Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-33920
Starting Date: June 1, 1973 Expiration Date: February 28, 1976
Goal: To determine the role of prostaglandins (PG) in mammary gland neoplasia.

Approach: Primary monolayer cultures of epithelial cells from normal and tumor-bearing mice to be used as the model system. Mammary cells of human origin will be utilized when they become readily available. Studies of PG synthesis in cultured cells, normal and neoplastic, from pregnant, lactating and tumor-bearing C3H mice to continue. Factors affecting PG synthesis, inhibitors and stimulators, will be examined. In collaboration with Dr. Maudsley studies of effects on cyclic nucleotide synthesis will be expanded. PG synthesis of fibroblast cultures will be determined.

Progress: Developed primary cultures of mammary epithelial cells into a viable and practical system for the study of PG synthesis of the mammary gland. Observations on PG synthesis in cultured cells have shown that arachidonic acid (20:4) is a more efficient precursor for PG production than eicosatrienoic acid (20:3). Dibutyryl cAMP was found to stimulate the synthesis of PGE_1 and PGF_{1a} in normal cells. There appeared to be a tissue dependent shift from stimulation of PGE at 48 hours to PGF at 72 hours and also a reduction of phospholipid and an increase in triglyceride content of the cells. C3H mouse mammary tumor cells have not been tested to date with dibutyryl cAMP. However the tlc patterns of tumor cells did not seem to differ significantly from normal cells. They were found to be unresponsive to LH, phospholipase A and drugs such as Naproxin and CBN. Initial studies have been carried out with fibroblasts from human lung utilizing the labeled precursor approach. Appreciable PGE synthesis was obtained.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $34,500

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acid Preparations on the Biological Properties of Mammary Carcinomas

Principal Investigators: Janet S. Butel, Ph.D., Florence Farber, Ph.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas 77025
Organization:

Contract Number: N01-CB-53904
Starting Date: April 1, 1975 Expiration Date: March 31, 1976
Goal: To determine the relationship between transfer of nucleic acid preparations into cells and possible changes in their growth potential.

Approach: The in-depth study of gene transfer into somatic cells will be undertaken to:

1) Determine favorable conditions for the uptake of foreign DNA by cells via different parameters as well as adding various facilitators with the purified DNA preparations.

2) Demonstrate the biological activity by the heterologous DNA using specific markers. a) HGPRTase activity; b) attachment of picornaviruses to cells after exposure to primate cell DNA.

3) Determine if transduction by SV40 pseudovirions is feasable and compare their efficiency for gene transfer to that done by direct DNA application.

4) Quantitate the intracellular fat of exogenous DNA in recipient cells.

5) Explore underlying mechanisms controlling gene expression.

The following cells will be utilized: Chinese hamster lung cells (CHL), mouse mammary cells, mammary tumor virus (MTV) cell lines, transformed cell lines.

Progress: New Contract

Significance for Cancer Research (NCP 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 8/6/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $90,000

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in Which They Influence Cellular Biochemistry

Principal Investigators: Kermit L. Carroway, Ph.D., Kurt E. Ebner, Ph.D.
Name/Address Performing Organization: Oklahoma State University, Stillwater, Oklahoma 74074, and University of Kansas Medical Center

Contract Number: N01-CB-33910
Starting Date: June 27, 1973 Expiration Date: June 26, 1976
Goal: To study composition and biochemical properties of normal and neoplastic mammary cell plasma membranes.

Approach: Use of several enzymatic procedures to prepare single cell suspensions from normal lactating mammary glands and tumors of rats. Cell types will be separated by differential centrifugation in various gradients and specific affinity columns whereby prolactin will be bound chemically to an inert support as Agarose or nylon. Use of radioactive glucosamine, fucose and leucine to assess repair of and labelling of cell membranes. Surface structures of normal and tumor membranes will be evaluated by disc gel electrophoresis, glycoprotein patterns, lectin binding, susceptibility to proteolysis, iodination with lactoperoxidase. The purity of isolated cell membranes will be assessed by biochemical and microscopic methods. Quantitative binding of prolactin to cell membranes will be determined.

Progress: Plasma membrane enriched fractions were prepared from lactating mammary tissue and R3230AC adenocarcinoma by fractionation of a tissue microsomal fraction. Plasma membranes were prepared from the 13762A ascites mammary adenocarcinoma. Isolated R3230AC cell suspensions were obtained by mechanical disruption and mild enzymatic treatment. Membranes isolated from these cells compare well to those from tumor tissue in terms of protein and glycoprotein distributions. Presence of galactosyltransferase activity in the plasma membrane preparations indicated Golgi contamination. Will attempt to eliminate the contaminant by subfractionation of the membranes. Conconavalin A inactivated 5-Nucleotidase by a direct action on this enzyme whereas mg^{++}-ATPase is activated by this cell surface effector. Prolactin receptor from rabbit mammary particles were solubilized and its purification by column chromatographic methods were tested. Isolation of prolactin receptor from rat tissue was complicated by its instability.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/8/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $100,000

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: R. David Cole, Ph.D.
Name/Address University of California
Performing Berkeley, California 94720
Organization:

Contract Number: N01-CB-43866
Starting Date: June 29, 1974 Expiration Date: June 28, 1976
Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones.

Approach: 1) Demonstrate hormone sensitivity in mammary epithelial cell line (NMuMG). 2) Observe hormonal effects on the program of chromatin assembly through the sequence of appearance and modification of chromosomal proteins. 3) Observe maturation of chromatin by characterizing 3 or 4 structural categories of chromatin fragments at different stages of chromatin maturation. 4) Compare all aspects of above with other mammary tumor cell lines to NMuMG. 5) Assay for prolactin receptors.

Progress: Progress was delayed because of arrival of required equipment and poor growth of the NMuMG cell line. The cell line failed to grow to confluence and was contaminated with PPLO and bacteria. Batches of sera were screened to search for factors contributing to the poor cell growth. Initial experiments performed prior to the cell growth problem showed lysine incorporation into all histones including five lysine-rich subfractions and into non-histone chromosomal (NHC) proteins. The patterns were unaffected by hormones. Phosphorylation into histones was not detected but some was detected into NHC proteins. Chromatin from several types of cells were sheared and fractionated into 3-4 species of fragments differing in melting temperature and sedimentation coeffecient from 20-200. There were modest variations among the fractions in protein content but little difference in the range of DNA molecular weights in each fraction. Low salt concentration denatures the chromatin at a level of organization higher than the subunit structure. The relative amounts of the 3-4 species of fragments varies with cell types.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $95,000

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: W. B. Dandliker, Ph.D.
Name/Address Scripps Clinic and Research Foundation
Performing La Jolla, California 92037
Organization:

Contract Number: N01-CB-43905
Starting Date: June 1, 1974 Expiration Date: Feb. 28, 1976

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones. Investigate possible competitive, cooperative or sequential effects in the binding of hormones to their specific cellular receptors of mammary tissue.

Approach: Fluorescent tagging of hormones, e.g., prolactin, growth hormone, insulin, estradiol, testosterone and purification of the fluorescent derivatives by chromatography. Equilibrium and kinetics of hormone binding to specific receptors is then monitored by changes in fluorescence polarization of the labeled hormones. Cellular receptors prepared from mammary tissues of pregnant rabbits and rat mammary tumor cell lines. Methods for preparing receptors include differential centrifugation, DEAE cellulose chromatography and affinity chromatography.

Progress: Fluorescence polarization methodology has been applied to the specific binding of prolactin and estradiol to receptors prepared from normal rabbit mammary tissue. Equilibrium measurements treated by Scatchard plots have shown that there are high affinity sites (K $\sim 10^9$ l mole^{-1}), as well as lower affinity sites (K $\sim 10^8$) for both hormones. Kinetic results on the interaction of prolactin with the microsomal fraction are consistent with a bimolecular reaction involving significant structural rearrangements during the reaction (not diffusion controlled). Stopped flow kinetic measurements on the reaction between estradiol and cytosol receptors show that at low temperatures, the reaction goes in two distinct steps separable in time. The second step may be the reaction found by others (utilizing sedimentation velocity methods) and which precedes translocation of the hormone-receptor complex to the nucleus.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/13/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $67,500

CONTRACT RESEARCH SUMMARY

Title: Studies on Basic Biological Events in the Pathogenesis of Mammary Cancer

Principal Investigator: Thomas L. Dao, M.D.
Name/Address Roswell Park Memorial Institute
Performing 666 Elm Street
Organization: Buffalo, New York 14203

Contract Number: N01-CB-23865
Starting Date: June 15, 1972 Expiration Date: June 14, 1976
Goal: Determine the role of hormones and other host factors in the pathogenesis of mammary dysplasia and neoplasia.

Approach: 1) Continue characterization of hyperplastic alveolar nodules (HAN) of rat mammary tissue by: cell kinetics consisting of cell proliferation rate *in vivo*, DNA synthesis, and cell cycle; karyotyping of chromosomes and their banding patterns. 2) Investigate the fate of HAN by: transplantation of individual HAN into subscapular fat pad of isologous recipient host and observe whether neoplastic transformation occurs; transplanting the whole mammary gland (with HAN) into the interscapular fat pad and following HAN growth; and, studying the incidence of HAN in aging rats and determining their natural history. 3) Determine the malignant potential of HAN *in vitro* by exposure to low levels of DMBA and transplanting *in vivo*; also *in vivo* experiments with whole mammary gland (with HAN) transplanted into isologous host and host treatment of DMBA (low level). 4) Study the effect of DMBA metabolites on the induction of HAN *in vivo* and *in vitro*.

Progress: Have successfully cultivated normal, neoplastic and HAN mammary tissue from Sprague Dawley rats for long periods. Developed a technique for identification of HAN *in vivo* so they can be removed for biochemical and transplantation studies. Hormone responsiveness of the three kinds of tissue have been compared biochemically using insulin, estradiol, progesterone and prolactin; the data suggest that HAN contain cell populations which are not in proliferation as compared to those of mammary gland or the tumor, therefore they do not support the concept that HAN is an intermediate step in the tumorigenesis of the mammary gland in the rat. DMBA-induced mammary tumors have been demonstrated to be of ductal origin. HAN transplanted into the interscapular fat pad develop either lobuloalveolar or ductal growth but not neoplasia. Biochemical characterization of HAN shows a lack of hormone dependence. One paper was published in 1974, two are in press.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $75,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Nature of Hormone Dependency in Breast Cancer

Principal Investigator: Eugene DeSombre, Ph.D.
Name/Address Performing Organization: University of Chicago
Chicago, Illinois 60637

Contract Number: N01-CB-23873
Starting Date: June 1, 1972 Expiration Date: May 31, 1976
Goal: To assess the role of hormones and specific hormonal receptors in the control of tumor growth.

Approach: Carcinogen-induced (DMBA) primary or transplantable mammary tumors of rats and appropriate control tissues are utilized. Major emphasis is to be placed on tumor RNA polymerase activity as affected by hormones, however, other projects on hormonal control of tumor growth and the role of hormonal receptors are to be continued. Major areas of study are: tumor RNA polymerase (I and II) activity; hormone receptors in apparently autonomous mammary tumors; interrelation of estrogen and prolactin receptors in tumors; transplantable, hormone-dependent mammary tumors; regulation of tumor peroxidase activity; and, cross reactivity of mammary tumor to antibody to estrogen receptor protein.

Progress: Before assaying for RNA polymerase stimulation by estrogen the tumor is classified as hormone-dependent (HD) or independent (HI) according to *in vivo* tumor response following castration. Assay is in a low salt Mg^{++} system and dependent on all 4 ribonucleotide precursors and is inhibited by actinomycin D. At 30°C RNA synthesis is linear for 5-10 minutes with no appreciable difference in product degradation in hormone-treated and control groups. Incubation of tumor homogenates with estradiol (E2) stimulates RNA polymerase I activity in hormone dependent tumors but not with receptor containing hormone-independent tumors. Requirement for E2 stimulation are receptor containing cytosol and HD tumor nuclei. Stimulation is temperature dependent occurring after 25°C. Anti-estrogen (C1628) which regresses HD tumors *in vivo* appears to block E2-induced stimulation *in vitro.* Some but not all (HI) tumors have significant concentrations of estrogen receptors. Mammary tumor peroxidase characteristic of HD tumors disappears after ovariectomy but reappears after E2 administration *in vivo*. Subcellular distribution is characteristic of tumor cell synthesis rather than cell uptake.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/12/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $112,000

CONTRACT RESEARCH SUMMARY

Title: Hormonal Requirements of Normal and Abnormal Human Mammary Tissue in Organ Culture

Principal Investigator: Joel J. Elias, Ph.D.
Name/Address University of California
Performing San Francisco, California 94143
Organization:

Contract Number: N01-CB-23861
Starting Date: June 19, 1972 Expiration Date: June 18, 1976

Goal: 1) To find conditions to maintain growth of normal, dysplastic and neoplastic human mammary tissue in organ culture. 2) To see whether morphologic classification of human mammary lesions can be improved so as to be of prognostic value.

Approach: Human mammary tissue from biopsies, mastectomies, and mammoplasties will be utilized for the following studies: 1) Electron microscopic, scanning electron microscopic and light microscopic preparations of normal and abnormal human mammary tissue to define histological, structural and surface characteristics. 2) Organ culture and eventually cell culture of material studied in (1). Analysis of conditions necessary for promoting growth. 3) Effects of hormones on organ cultures studied with EM, SEM and LM. Correlation with biological behavior in vivo.

Progress: Organ culture techniques were developed for human breast carcinomas to be maintained up to 20 days in medium 199. Tritiated thymidine labelling and autoradiography showed minimal DNA synthesis in explanted carcinoma cells. Attempts to stimulate DNA synthesis using various hormone combinations and concentrations, placental cord serum, and serum from the patient were unsuccessful. Attempts to stimulate ductal or alveolar growth and/or secretion in dysplasias in culture by using various combinations of insulin, cortisol, human placental lactogen, etc., have not been successful. Progress increased in recognizing surface features of epithelial cells in human mammary dysplasias, fibroadenomas and carcinomas by scanning electron microscopic (SEM) methods. Tissue from biopsy or mastectomy from 43 women were studied by SEM to date. Analysis of specimens from the first 13 women easily identified infiltrating ductal carcinoma cells and moreso recognized that duct epithelium within breasts containing benign lesions differed from that within carcinomatous breasts. Characteristics of duct cells in carcinomas are: (1) partitioning of surface microvilli into small groups of 3 or more clumped together at the tips; (2) microvilli from adjacent cells touching at their apices; (3) prominent clump of microvillis-like projections in the center of apical surface. Ducts from histologically "normal" areas of the breast and as far as 7 inches from biopsy site showed these structural characteristics.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/9/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $80,000

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Mammary Epithelial Cell Plasma Membrane

Principal Investigator: Grant Fairbanks, Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-33908
Starting Date: June 1, 1973 Expiration Date: Feb. 28, 1976
Goal: To develop a new method of plasma membrane isolation for application to normal and neoplastic mammary cells.

Approach: Non-permeating plasma membrane (PM) labelling reagents will be used to endow surfaces of intact, viable cells with foreign specificities to be the basis for affinity isolation of plasma membrane vesicles in high yield and purity. The ascites 13762A rat mammary adenocarcinoma will serve as a model and the methodology will be tested against human cell lines; HBL-100 from normal breast tissue, MDA-MB-175, and BT-20 both from infiltrating duct carcinomas; MDA-MB-231 from an intraductal carcinoma.

Progress: Materials for two new methods of plasma membrane isolation have been assembled. These are based on reagents containing a cleavable disulfide bond and N-hydroxysuccinimide (NHS) esters reactive with protein amino groups. Synthesis of the first radiolabeled, cleavable protein cross-linker was accomplished. This (^{35}S)-bis NHS ester disulfide was condensed with pyridoxamine phosphate (PMP) to yield a new membrane labeling and isolation reagent. An immobilized apoenzyme for selective adsorption of membranes derivitized with the PMP-NHS reagent was prepared in pilot studies and shown to bind the reagent. Condensation of the cleavable cross-linker with amino alkylated glass yielded NHS-activated beads that couple to cell surfaces. The stable (but reversible) attachment of plasma membranes to beads will be the basis for their isolation free of intracellular membranes. Studies of ^{3}H-poly(U) interactions with tumor cell surfaces suggest that polynucleotide homopolymers will not be useful as isolation reagents, but that their high affinity for certain surface sites may be exploited in plasma membrane subfractionation.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $40,500

CONTRACT RESEARCH SUMMARY

Title: Cultivation of Normal and Malignant Human Mammary Tissue: Hormone Dependent Susceptibility of Mammary Tissue to Transformation

Principal Investigator: Edwin V. Gaffney, Ph.D.
Name/Address Pennsylvania State University
Performing University Park, Pennsylvania 16802
Organization:

Contract Number: NO1-CB-33898
Starting Date: June 28, 1973 Expiration Date: March 27, 1976
Goal: Isolate and maintain pure cultures of human epithelial cells derived from normal and malignant breast tissues as well as from milk. To determine factors which enhance or depress proliferation of malignant cells in vitro. To establish if certain lactogenic hormones will predispose normal breast cells to malignant alterations.

Approach: In vitro culture study of normal and malignant cells. Single cell suspensions of normal and malignant breast tissue prepared with collagenase and trypsin. Cells separated by sedimentation at unit gravity in bovine serum albumin gradients. Use of polyacrylamide gel to isolate epithelial cell types without fibroblast overgrowth. Isolate normal or pre-malignant epithelial cells from aspirated human milk samples. Hormone effects on malignant and normal cells.

Progress: In vitro cultures have been established from tissues obtained from specimens at autopsy, reduction mammoplasty and lactating human breasts. Pure epithelial cultures were selected by seeding suspensions of polyacrylamide gels followed by transfer to plastic or glass surfaces. All cultures subjected to lactogenic hormones had increased DNA synthesis and phosphoproteins. Cell cultures derived from tissues of patients of various age groups demonstrated differences in formation of tumor antigen following SV40 infection in presence of prolactin and/or estradiol. Low concentrations of spermadine enhances whereas high concentrations inhibits DNA synthesis. Hormones are required in the media for cancer cells to replicate in vitro. Cell cultures always establish from tumors which metastasize but rarely establish from samples of small primary tumors. Two epithelial and two fibroblast-like cell lines were initiated from human milk samples obtained from 250 nursing mothers. Initial chromosomal mode studies were done on two lines and one cell line was inoculated into nude-athymic mice to determine tumorigenicity.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/27/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $67,500

CONTRACT RESEARCH SUMMARY

Title: Studies of Isoenzymes in Normal, Preneoplastic and Neoplastic Breast
Tissues as an Approach to Define Unique Properties of Neoplasia

Principal Investigator: Russell Hilf, Ph.D.
Name/Address Performing Organization: University of Rochester
Rochester, New York 14642

Contract Number: N01-CB-33880
Starting Date: May 21, 1973 Expiration Date: Feb. 20, 1976

Goal: To examine multiple molecular forms of glucose-6P-dehydrogenase G6PD and lactate dehydrogenase LDH to characterize normal, preneoplastic and neoplastic mouse mammary tissue.

Approach: Molecular forms of G6PD and LDH to be examined in normal mouse mammary tissue (pregnancy, lactation, parturition) and in preneoplastic and neoplastic tissue exposed to the same hormonal milieu *in vivo*. Determine sulfhydryl content of tissues, with emphasis on reduced glutathione, to seek correlations with G6PD profiles in normal and abnormal tissue. Establish the molecular weights of G6PD-III, II and I, using methods such as that of Hedrick-Smith. Purify and characterize G6PD from glands of lactating mice. Use G6PD as an antigen to induce antibodies. Expand studies on G6PD forms in tissues of mice possessing NIV and MTV.

Progress: Enzyme activities (per mg. protein) in hyperplastic alveolar nodules (HAN) from virgin mice were more like those of mammary glands (MG) from pregnant mice than mammary tissue (MT) from lactating mice. However, when expressed per mg/DNA they were lower in HAN than in MG from pregnant or lactating mice. In transplanted carcinomas activities resembled those in HAN more so than those in spontaneous tumors or tumors arising from transplanted HAN. Glutathione reductase (GR) activity was elevated in HAN and tumors from virgin mice as compared to MG, whereas the α-glycerolphosphate dehydrogenase activity was lower in both abnormal tissues. HAN from lactating mice showed increase in RNA levels and a decrease in LDH. GPI and GR compared to HAN from virgin mice which suggests preneoplasia retains some ability to respond to altered hormonal milieu. MT from lactating Balb/c mice showed up to 50% of the slow migrating G6PD-III which is absent in glands from pregnant mice, HAN, and carcinomas. All tissues possessed the faster migrating G6PD-II, 85% in pregnant MG. The fastest migrating G6PD-1, up to 35% of total activity in HAN and neoplastic tissue, but only 15% in glands from pregnant mice and absent in lactating gland. Addition of DTT to tissue did not alter G6PD activity but increased relative activity of G6PD-II and GPD-1. Molecular weight estimations were 118,000 for G6PD-II and 260,000 for G6PD-III. Progress has been achieved in distinguishing normal from preneoplastic and neoplastic mammary tissue.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 6/21/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $27,000

CONTRACT RESEARCH SUMMARY

Title: Isolation of Prolactin Cells from Human and Rat Adenohypophysis: A New Approach to the Study of Control of Prolactin Secretion in Relation to Mammary Cancer

Principal Investigator: Wesley C. Hymer, Ph.D.
Name/Address Performing Organization: The Pennsylvania State University
University Park, Pennsylvania 16802

Contract Number: N01-CB-23863
Starting Date: June 28, 1972 Expiration Date: March 27, 1976
Goal: To isolate mammotrophs from human and rat adenohypophysis, to cultivate them and study their effects on mammary tissue, normal and neoplastic.

Approach: The following materials will be used: 1) Human adenohypophysis from autopsy and surgical material. 2) Rat adenohypophysis from normal, lactating, estrogen treated and pituitary mammotropic tumor bearing females. The experiments will include: 1) Cell dissociation. 2) Cell separation by sedimentation at 1G in BSA gradient chamber (NIH model). 3) Culture of separated cells alone and in co-culture with normal and malignant mammary cells. 4) Prolactin radioimmunoassay.

Progress: Rat co-culture experiments showed: 1) In 39 experiments 34 (88%) showed elevated levels of prolactin released by pituitary cells in co-culture with mammary cells versus that from an equivalent number of pituitary cells alone. Twenty-two of the 34 experiments were statistically significant($p<.05$) 2) Pituitary cells of lactating rats respond better than those from untreated ones. 3) Separated mammary fibroblasts or epithelial cells gave a good response but cells from parotid, kidney, pineal, or mammary fat did not. 4) β-glucuronidase, PGE, etc.,stimulated protein release only after 7 days exposure. The human co-culture experiments showed: 1) Of 21 experiments 16 (76%) showed elevated prolactin release when co-cultured with human mammary cells and 56% of these experiments were statistically significant. 2) Normal cells from reduction mammoplasty or cells from malignant tissue gave a consistant response whereas established cell lines as ALAB showed fewer responses. The results may be important in defining the hypothalamic pituitary-mammary gland axis. Some samples of human pituitary cells grew in aggregates, e.g., one case the prolactin yield was approx. 0.5 ug prolactin/day/75,000 cells seeded during 44 days. Pituitaries from human breast cancer patients showed hypertrophied mammotrophs. Since May 1974,14 human pituitary glands were obtained: 6 from patients with breast cancer, 2 with galactorrhea, 3 with acromegaly, 2 with chromaphobe adenomas, and 1 diabetic.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Project Officer, D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/3/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $61,500

CONTRACT RESEARCH SUMMARY

Title: Studies on the Prevention of Metastasis in Mammary Cancer

Principal Investigator: Untae Kim, M.D.
Name/Address Performing Organization: Roswell Park Memorial Institute
Buffalo, New York 14203

Contract Number: N01-CB-23864
Starting Date: June 15, 1972 Expiration Date: June 14, 1976

Goal: Determine the mechanism of breast cancer cell dissemination in relation to plasma membrane properties and host immune states, and to develop clinically applicable procedures and rationale for prevention of metastasis.

Approach: Utilizing spontaneously metastasizing (M) and non-metastasizing (NM) mammary carcinomas of W/Fu rats, the following studies are envisioned: 1) Biochemical analysis of glycoprotein shedding mechanism. Extending studies of cell surface glycoproteins and antigens as well as adenyl cyclase levels in tumor cells and presence of lysosomal enzymes. 2) Host immune responses to M and NM tumor cells. Patterns of spleen and thymus cell migration in the same tumor host will be compared to establish the role of T and B lymphocytes in the immune escape mechanism. 3) Establishment of biochemical guidelines for single vs. radical mastectomy. Human tissue from biopsy and surgery to be examined for plasma membrane associated enzymes and data correlated with degree of metastases. 4) Experimental therapeutic procedure will be examined for effects on metastasis. 5) Immunology of prolactin receptors in tumors will be determined in collaboration with Dr. W. Dandliker.

Progress: A publication is in press on the spontaneously M and NM rat mammary carcinomas, the tumors and their plasma membranes have been characterized in terms of their antigenicity, glycocalyx, 5-'nucleotidase activity and presence of a membrane bound antigen thought to be the tumor specific transplantation antigen. The 3':5' cyclic monophosphate phosphodiesterase enzyme activity was found to be 2.5 x higher in NM tumors. Steady state levels of cAMP were 1.3 to 2.0 x higher in M tumors. Biochemical properties of phosphodiesterases of M vs. NM tumors were compared. No major differences were seen except a 2 x greater rate of purine nucleotide hydrolysis in NM tumors. Spleen cells from NM tumor hosts migrate as well as normal spleen cells using a standard MIF test. Spleen cells from M tumor hosts were inhibited by 40 to 60%. Incubation of host spleen cells with extracts from their respective tumors reversed this observation. Characteristics of the immunogenicity of the size dependent-self-curing DMBA-4 mammary tumor were explored.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $105,000

CONTRACT RESEARCH SUMMARY

Title: Role of the Stroma in the Growth of Neoplastic and Preneoplastic Lesions of the Mammary Gland

Principal Investigator: Peter O. Kohler, M.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas 77025
Organization:

Contract Number: N01-CB-43907
Starting Date: June 10, 1974 Expiration Date: June 9, 1976
Goal: Determine the relationship between stromal and other mesenchymal cells on growth and differentiation of normal and malignant epithelial cells from the mouse mammary gland.

Approach: Develop and utilize mammary cell culture systems in which normal and malignant epithelial cells can be co-cultured in presence of mesenchymal cells either in direct contact or separated by a filter to permit passage of only cell products. Characterize cloned cell strains from mouse HAN and mammary tumor, as well as rat mammary tumor with regard to growth rates, hormone receptors, precursor incorporation into DNA and protein, and differentiation using light microscopy. Study the effect of stromal cells from various sources as embryonic mouse cells, defatted stroma from mammary fat pads, and WI-38 cells on the growth of the characterized cells listed above in co-culture or in separated filter and perfusion chamber culture. Study the specificity of various mammary fat depots in regulating growth of normal and pre-neoplastic epithelial cells.

Progress: Isolated and cloned the following: 1) Several strains of HAN from D-2 mice of which 2 exhibited estradiol binding; 2) Six strains of mammary cells from D-2 tumor in Balb/c mice and propagated in culture for 2 months of which 4 exhibited estrogen binding; 3) Six strains of mammary tumor from primary induced tumors in Sprague-Dawley rats. Co-cultivated stroma with HAN cells and mouse tumor cells and evaluating the growth and estrogen binding activity. Culturing cells on millipore and nuclepore filters in a perfusion chamber and assessing the effect on growth and differentiation. Developed a method to obtain single cell suspensions of epithelial cells from mouse tissues, virgin, pregnant and HAN. These produced a yield of 12.8×10^6, 20.2×10^6, and 57.5×10^6 cells/gm. mammary tissue wet weight respectively. Initiated a long-term experiment involving the effect of a chemical carcinogen on the ability of stroma to influence growth and tumor potential of nodule tissue. Six cloned tumor lines and one nodule line developed in vitro were transplanted into syngeneic mice to assess their neoplastic state.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Richard A. Knazek, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/15/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $87,000

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Name/Address Performing Organization:	Klaus Kratochwil, Ph.D. Institute for Molecular Biology Salzburg, AUSTRIA

Contract Number: N01-CB-33883
Starting Date: June 29, 1973 Expiration Date: March 28, 1976

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland.

Approach: Determine whether sensitivity of mammary tissue to androgen exists during the entire developmental period or is it acquired at a specific time. Mammary gland buds of various developmental stages are exposed to testosterone *in vitro*. Hormone binding of mammary tissue to be studied in its correlation with acquisition of hormone responsiveness. Concentrate in the year ahead on: finding the basis for transient androgen responsiveness of the embryonic mammary gland rudiment and on identifying primary target tissue for testosterone; analyze subsequent processes, with special attention to tissue and cell interaction; and complete the morphological study of the response itself and obtain data on the fate and of role of tissue interface material. e.g. basal lamina, in the androgen response.

Progress: Established that the morphological response to testosterone of explanted mammary gland rudiments is identical to that found in the male fetuses. This made it possible to work out a sequence of events on explants exposed to testosterone for precisely known periods. The outstanding feature is perhaps the disappearance of the basal lamina before the onset of necrosis. A difficult technical problem in the analysis of tissue interaction, the enzymatic separation of mammary epithelium from all adhering mesenchymal cells has been overcome. This should make it possible now to identify the primary target tissue for testosterone in Tfm-wild type tissue combinations (Tfm mice). Such identification would be a major step forward.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $30,000

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which they Influence Cellular Biochemistry

Principal Investigator: Zoltan Lucas, M.D.
Name/Address Performing Organization: Leland Stanford Jr. University
Stanford, California 94305

Contract Number: NO1-CB-33905
Starting Date: May 24, 1973 Expiration Date: May 23, 1976
Goal: To study natural mechanisms which impair the host's cellular immune response to syngeneic mammary tumors.

Approach: Determine the immune response to several rat mammary tumors (induced or spontaneous) in syngeneic animals in which the tumors grow progressively. Determine if tumor growth is associated with factors blocking cellular immunity (CI), investigating both serum (tumor membrane antigen, enhancing or "blocking" antibody, antigen-antibody complexes, and "anti-antibody-to-tumor antigen)" and cellular (suppressor T- and suppressor B-cells) factors as well as non-membrane tumor products non-specifically inhibiting CI. Determine the kinetics of the blocking factors following syngeneic tumor implantation. Determine if equivalent factors arise following allogeneic mammary tumor or normal tissue grafts.

Progress: Fischer rats injected with ascitic syngeneic mammary tumor 13762A develop cell mediated cytotoxicity that peak on day 8 and then decrease to variably low levels between days 10 and 14. Memory cells for the mammary tumor are first detected three days after innoculation and peak on days 5-8. Subsequent detection depends on the techniques used to measure them. If they are detected by assays involving direct transfer from animal to sensitizing monolayers of tumor cells, memory cells are not detected 10 days after immunization. If, however, they are first adaptively transferred to irradiated syngeneic animals, and tested for activity five days later, they persist throughout the entire period of primary immunization. This suggests that animals 10 days after immunization contain two populations of cells, memory cells for tumor and suppressor cells interfering with the conversion of memory to cytotoxic effector cell. A direct assay for suppressor cell activity was developed confirming the above hypothesis.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $100,000

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of 3', 5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia

Principal Investigator: David Maudsley, Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-33919
Starting Date: June 1, 1973 Expiration Date: Feb. 28, 1976

Goal: To study the role of cyclic adenosine 3',5'-monophosphate (cAMP) in the mammary gland tissue, normal and abnormal, and to establish whether the incidence of mammary tumors alters cAMP metabolism.

Approach: Originally used freshly isolated and primary cultures of mouse epithelial cells, future studies will utilize established human breast cells, normal and abnormal. Compare findings with isolated cell suspensions and primary cultures of mouse tissue with response of established human cells. Evaluate the effect of prostaglandin precursors on cAMP production. Evaluate the possibility that peptide and steroid hormones interact through a common binding site in activating adenyl cyclase. Manipulating levels of cAMP in cells to define the role of this component in cell growth regulation.

Progress: Prostaglandin E_1 (PGE_1), epinephrine (EP) and cholera enterotoxin (CT) stimulated cyclic AMP (cAMP) levels in isolated cell suspensions of mammary tissue from mid-pregnant C_3H mice whereas insulin, prolactin, ACTH, and glucocorticoids had no effect. PGE_1 response occurred in 30 seconds and completed by 20 minutes. CT response occurred within 10 minutes and reached maximum at 90 minutes. Response of all 3 agents was enhanced by theophylline, PGE_1 combined with EP or PGE_1 with CT were additive which suggested stimuli operate different mechanisms or stimulate different cell types. In isolated cell suspensions CT was most potent, followed by PGE_1 and EP lowest whereas in primary monolayer cultures PGE_1 gave the lowest. Study suggested a component in cell suspension in addition to epithelial cells was responsive to PGE_1 stimulation. Cell suspensions from rat mammary tumor systems DMBA primary, 13762, and R-35 all had similar elevated cAMP levels in presence of CT, PGE_1 and EP but not to ACTH, prolactin or LH. The ascites 13762A responded to CT and EP but was markedly reduced to PGE_1.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $57,000

CONTRACT RESEARCH SUMMARY

Title: Protein Nucleic Acid Interactions in Transcription Controls of Normal and Malignant Cells

Principal Investigator: Kenneth S. McCarty, Ph.D.
Name/Address Duke University
Performing Durham, North Carolina 27710
Organization:

Contract Number: N01-CB-23857
Starting Date: June 29, 1972 Expiration Date: March 28, 1976

Goal: To elucidate the role of proteins of the mammary cell nucleus in the expression and regulation of genetic information of normal and malignant cells.

Approach: Improving techniques for the isolation of chromatin and the extraction of acidic nuclear proteins. DNA-protein rebinding techniques to be used with isolated nuclear protein to determine the comparative affinities for phosphorylated and dephosphorylated acidic chromosomal proteins. Techniques of chromatin reconstruction and transcription to be used to determine effect of hormones. Chromatin interactions with phosphorylated and dephosphorylated proteins. Examine RNA synthesized as a result of prolactin exposure in intact cells and isolated nuclei. Determine inductive capacity of hormones on mammary gland polyamine biosynthesis. Fischer/344 rat mammary tissue, normal and neoplastic (13762 adenocarcinoma) is being utilized in addition to studies of C_3H mouse mammary tissue.

Progress: Developed techniques for preparation of nuclei, histones and chromosomal proteins from Fischer/344 rat mammary gland. Techniques were modified for mammary gland tissue compared to other tissues as spleen, brain, liver, etc. Homogenization with hexylene glycol was essential for mammary gland fractionation and isolation of pure nuclei. NP 40 was required to strip the rough endoplasmic reticulum and remove nuclear membrane components assuring no contamination of acidic chromosomal proteins. Nuclei were fractionated with a Bronson sonifier into 3 components; soluble fraction chromatin supernatant, membrane-interphase, nucleolar-pellet. The chromatin was dissociated utilizing gradients of lithium chloride, 5 M urea, TOC, into histones and acidic chromosomal proteins. Cetyltrimethylammonium bromide(CTAB) was used to remove DNA and further separate RNA from DNA. Developed an apparatus for mass culture of surface contacted cells which are essential for the study of chromosomal protein alterations. The cell suspension cultures in contrast to surface contact cultures fail to synthesize specific proteins and do not respond to hormones. The apparatus will be available commercially as the Bellco Glass Apparatus-McCarty Cytosynchronator. Several papers were published in this contract period by McCarty and colleagues.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 7/25/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $45,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine Induced Breast Cancer Regression

Principal Investigator: Name/Address Performing Organization:	William L. McGuire, M.D. University of Texas Medical School San Antonio, Texas 78284

Contract Number: NO1-CB-23862
Starting Date: June 1, 1972 Expiration Date: May 31, 1976

Goal: To identify those breast cancer patients whose disease will respond to hormonal manipulation and to obtain new knowledge of the hormonal controls on tumor growth.

Approach: Development of predictive procedures for hormone dependence in human breast cancer by extending estrogen receptor (ER) clinical studies into new areas, by simplifying ER assays, and by investigating other steps in hormonal control to gain additional information on hormone responsiveness. In addition to ER determinations on human breast cancer tissue will undertake studies of prolactin (Prl R) and progesterone receptors (PgR).

Progress: Over 226 human breast tumors were for ER; 50 had objective response to endocrine therapies. Tumors without ER had 100% negative response to therapies whereas ER positive tumors had 60% objective regression. Methodology for determining ER was improved by use of thiol reagents and temperature control. Solid phase ligand exchange assay was developed to determine cytoplasmic ER in the presence of endogenous estrogen. Developed a PgR assay in human tumor cytosols since PgR is produced in response to estrogen in target tissues. Of 13/13 ER- tumors 100% lacked PgR whereas 11/20 or 55% of ER+ had PgR. Where clinical response can be compared directly, 3/3 ER+ PgR+ tumors had objective regression while 2/2 ER+ PgR- failed to respond. Initiated studies on role of Prl R in hormone dependence of mammary tumors. Binding of 125_1 prolactin was similar in normal rat mammary gland, a dependent ER+ tumor, and one autonomous tumor with low ER. Prl R disappeared from the female rat liver after hypophysectomy but was restored by injection of prolactin; autoregulation is a new concept in polypeptide hormone physiology. The anti-tumor agent Estradiol Mustard (EM) has low affinity for ER *in vitro* and a long time lag before effects on ER *in vivo* were observed which suggests a hydrolysis product and not EM is responsible. Assays for ER and Prl R done on tissues and cell lines for other investigators.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/24/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $135,000

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Beppino C. Giovanella, Ph.D.
Name/Address Performing Organization: The Stehlin Foundation
Houston, Texas 77002

Contract Number: N01-CB-33856
Starting Date: February 1, 1973 Expiration Date: Jan. 31, 1976

Goal: To ascertain whether a thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential and hormone responsiveness.

Approach: A colony of thymusless-nude Swiss mice has been developed and the good health of the animals permits their use in long-term experiments. Approximately fifty human mammary carcinomas will be transplanted each year, each tumor sample is transplanted into at least 3 females and 1 male. Tumor fragments immediately after removal are inserted subcutaneously into two fat pads and on the back. When enough material is available, it will be transplanted also in splenectomized and/or AL(B)S treated mice and in ones older than 6 months. After transplantation the host will be followed until death. If the tumor grows, it will be re-transplanted for 3 generations if feasible. The transplanted tumor fragment will be characterized by a pathologist. The morphology and karyotype of the growing mass will be compared with the original fragment.

Progress: The colony has been expanded to the point of 1,500 and 1,600 mice on experiment. Average life expectancy is more than a year and some of them are 2 years and older. Seventy primary and metastatic human breast carcinomas and 10 cell lines from human breast cancers have been transplanted in 624 nude-thymusless mice. Of 54 breast cancers transplanted directly from the patient and observed for 90 days or more, 27 grew slowly. Many regressed, some reappearing after months of latency. Four tumors showed indefinite growth between 6 months and a year. Two of the 4 were mucinous cell carcinomas, one an intraductal carcinoma and the fourth an infiltrating duct cell. A fifth primary carcinoma grew rapidly in nudes after a short latent period; this infiltrating duct cell carcinoma came from a young, premenopausal woman. Eight of ten human breast cancer cell lines grew vigorously in the nudes, they progressed to aggressive, invasive tumors which can be serially transplanted in nude mice. Two of the lines metastasized to lymph nodes and lungs. Two other cell lines exhibited preferential growth in males, two others in females.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 10/27/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $116,300

CONTRACT RESEARCH SUMMARY

Title: Studies to Determine the Pathological History of the Mammary Gland in Pseudohermaphroditic Rats and Mice

Principal Investigators: Susumu Ohno, D.V.M., Ph.D.
Name/Address James Kan, Ph.D.
Performing City of Hope Medical Center
Organization: Duarte, California 91010

Contract Number: N01-CB-33907
Starting Date: June 27, 1974 Expiration Date: June 26, 1976
Goal: To study the underlying mechanism of the inhibitory action of testosterone on mammary gland development by the use of the X-linked Tfm (testicular feminization) mutation of the mouse.

Approach: Study the role of 7,12-Dimethylbenzanthracene (DMBA) on hypothalamic function as to cause of mammary tumor development by: 1) Establishing conditions to restore estrus cycles to 100% of masculinized Tfm/+♀ mice. 2) Determining if DMBA demasculinized females in persistent estrus develop mammary carcinomas at the same rate as DMBA treated females in normal cycles. 3) Exploring if DMBA makes the hypothalamic center supersensitive to positive feedback by estrogen. 4) Using tritiated DMBA determine whether: it concentrates in the central nervous system of the hypothalamic region; and it has a high binding affinity to androgen or estrogen receptor proteins in this region.

Progress: In Tfm $(0^{hv})/+(0^{+})$, Sxr/+♂ sex reversed Tfm heterozygous males androgen inhibition of nipple formation is all or none i.e., mice have either all female-type nipples or male-type nipples. Testosterone suppression of lactation was found in 30% Tfm $(0^{hv})/+(0^{+})$♀ which resisted daily administration of 2 mg of testosterone and was also all or none. Testosterone's action on mammary gland cells is minimal and acts mainly by inhibiting pituitary prolactin release via the hypothalamic-pituitary axis. That serum prolactin levels were maintained during 2nd and 5th day of lactation but by 12th and 20th day levels were reduced showed evidence that testosterone exerted a negative feedback inhibition. 5α-Dihydrotestosterone was ineffective on lactating Tfm/+♀ mice. DMBA has a powerful effect on hypothalamic-pituitary axis. A single 5 mg dose DMBA given masculinized Tfm/+♀ in persistent estrus demasculinized the neonatally imprinted hypothalamus and restored estrus cycles. First ovulation appeared at 4 hours and the second on the 7th day after DMBA and five consecutive estrus cycles were observed in a few mice.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/10/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $100,000

CONTRACT RESEARCH SUMMARY

Title: Study of Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: William Pearlman, Ph.D.
Name/Address University of North Carolina
Performing School of Medicine
Organization: Chapel Hill, North Carolina 27514

Contract Number: N01-CB-53905
Starting Date: April 1, 1975 Expiration Date: March 31, 1976
Goal: To search for the presence of steroid hormone receptors in human and animal milk.

Approach: Raw milk from humans, cows and rats will be obtained at early and late stages of lactation, centrifuged and exposed to activated charcoal to remove endogenous steroid hormones in order to:

1) Determine the steroid-binding activity particularly with respect to progesterone, cortisol, 17β-estradiol, and testosterone.

2) Determine the effect of administered prolactin and cortisol on the levels of the steroid-binding activity of milk from lactating rats.

3) Subject whey proteins to various fractionation procedures so that protein components responsible for specific steroid-binding activity may be purified and partially characterized by physicochemical methods.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 7/24/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $82,700

CONTRACT RESEARCH SUMMARY

Title: Role of Prolactin in Breast Cancer

Principal Investigators; Olof H. Pearson, M.D.
Name/Address Jack Pensky, Ph.D.
Performing Case Western Reserve University
Organization: Cleveland, Ohio 44106

Contract Number: N01-CB-23859
Starting Date: June 26, 1972 Expiration Date: June 25, 1976
Goal: To find specific receptor sites for prolactin in breast cancer tissue which might predict hormone responsiveness of individual breast tumors.

Approach: Human and animal mammary tumor tissue for the following studies: 1) Isolate human prolactin (PRL) and develop specific immunoassay for human PRL; 2) Measure human PRL levels in serum and other biological fluids in breast cancer patients; 3) Detect and quantify prolactin receptors in membrane fractions of mammary tumors of animals and humans.

Progress: Prolactin (PRL) receptors were measured in DMBA induced rat mammary cancer and female rat liver. A significant correlation exists between presence of PRL receptors and hormone dependence in DMBA tumors. Liver PRL receptor levels increased after administration of ovine PRL or estradiol (E). PRL receptor levels in DMBA tumors are not affected by PRL or E in intact rats, but decrease after oophorectomy (OVX) and resynthesis after E administration. Estrogen receptors (ER) were measured in rat uterus and mammary tumors. One day post OVX ER doubled in uterus and tumor and remained high in uterus. Similar results were found 3 and 5 days post hypophysectomy (hypox). In tumors ER increased 3 days post OVX but then declined. Ovine PRL or E given before or after OVX did not affect ER in uterus. In intact animals ovine PRL did not affect tumor ER but ovine PRL or E given post OVX restored tumor ER levels. Therefore, PRL receptors are controlled by the pituitary but ER levels are a function of tumor or uterine cells. Human mammary tumors have low PRL receptors. A good correlation exists between presence of ER in human breast tumor cytosol and response to hypox. Lergotrile mesylate suppressed PRL secretion in 12 patients with metastatic breast cancer but did not induce remissions compared to hypox.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/18/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $85,000

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Dwight R. Robinson, M.D.
Name/Address Massachusetts General Hospital
Performing Boston, Massachusetts 02114
Organization:

Contract Number: N01-CB-33918
Starting Date: June 30, 1973 Expiration Date: March 29, 1976

Goal: To determine whether there are differences in prostaglandin (PG) synthesis by normal and neoplastic human breast tissue. To investigate the role of prostaglandins in growth and differentiation.

Approach: Production of PGE and PGF by benign and neoplastic human breast tissue will be measured. Surgical specimens are maintained in short term organ culture and the media analyzed for PGE, PGF and tissue and media for cyclic AMP by radioimmunoassays (method of Levine for PGS). Comparisons will be made between PG and cAMP between normal breast tissues, cystic disease, fibroadenomas and carcinomas. Correlations between these data and the clinical properties of the malignancies will be attempted. PG production and cAMP levels to be measured in culture medium of 2 clonal tissues--rat mammary tumor and ovary, a correlation will be sought between PG production and initiation or arrest of growth. These lines and synovial fibroblasts will be a reference point for studies of human material based on previous results. The effect of indomethacin, colchicine and other drugs on PG and cAMP production will be investigated.

Progress: Media from over 40 explants of tissues as breast cancer, normal breast tissue from reduction mammoplasties and apparent normal tissue surrounding breast tumors, fibrocystic disease, and fibroadenoma were analyzed for prostaglandin (PG). Breast cancer tissue had 10-fold more PGE than cystic disease tissue. Isolated cells from collagenase digested tissues are less prominent than minced tissues suggesting that orientation of cells in organ culture and intact tissue are related in some way to prostaglandin output. Indomethacin added to the incubation medium gave a >90% inhibition of PG production in all tissue studied. Incubation of organ cultures in presence of colchicine gave a 10-fold stimulation of PG in malignant, and benign tissues and tissue from other sources which suggests that microtubular structures are important in regulation of PG biosynthesis.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/4/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $51,750

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces and Influence Cellular Biochemistry

Principal Investigator: Arnold D. Rubin, M.D.
Name/Address Performing Organization: Mount Sinai School of Medicine
New York, New York 10029

Contract Number: NO1-CB-33912
Starting Date: June 28, 1974 Expiration Date: June 27, 1976
Goal: Analysis of the effects of lymphocyte effector molecule mammary cell interaction.

Approach: To purify the reversible inhibitor of lymphoid cell growth from SKBR-3 human breast cancer cell line, and to delineate the mechanism which produces the effect and to examine its potential to prevent antigen stimulated growth. Other breast cancer cell lines will be examined for inhibitor. Analyze lymphocyte growth in response to antigens extracted from the cell lines and from extracts of tumor tissue.

Progress: Establishment of epitheliod cell lines from breast tumor tissue encountered many difficulties, thus studies were restricted to established cell lines SKBR-3, GL-11 and HBT-3 which were developed from human biopsy materials. Characterization of these cell lines showed that only the SKBR-3 line represented a non-HeLa entity whereas the other two were contaminated with HeLa cells. Electronmicroscopic studies showed SKBR-3 had fibroblastic qualities. These cells developed an increase in RNA synthesis after exposure to insulin a quality not observed with GL-11 and HBT-3. SKBR-3 cell line showed an increase in lactose synthetase activity following exposure to insulin, hydrocortisone and prolactin. SKBR-3 line released a variety of humoral agents into the growth medium including a migratory inhibitory factor and lymphotoxin. The cells contained a powerful but reversible inhibitor of lymphoid cell growth which was 25 times more effective than any other lymphoid extract observed to date. Initiated studies on the blastogenic response of peripheral lymphocytes to antigens derived from breast cell lines from 4 normal persons, 4 patients with benign breast lesions and 4 with breast cancer. Lymphocytes from all patients showed a good response to extracts from the SKBR-3 line when only 3 of 4 responded to extracts from breast cancer tissue.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/30/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $80,000

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of 3', 5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia

Principal Investigator: David F. Scott, Ph.D.
Name/Address Medical College of Georgia
Performing Augusta, Georgia 30902
Organization:

Contract Number: N01-CB-33909
Starting Date: June 30, 1973 Expiration Date: June 29, 1976
Goal: To determine the involvement of 3', 5'-adenosine monophosphate (cAMP) in the proliferation of normal and neoplastic mammary tissues.

Approach: Adipose, ductal and alveolar cells of normal and developing mammary gland and neoplastic cells of mammary tumors will be isolated. The activities of adenylate cyclase and phosphodiesterase will be measured in homogenates of whole glands, isolated cells, mammary gland explants, and cultures of each cell type. The hormonal regulation of cAMP levels will be studied using mammary gland explants and isolated cells. Mice of the A, Balb/c and C3H strains will be used first, human tissue later. Compare growth rates and cAMP levels in tumors and normal cells. The main objective is to understand the mechanism(s) by which cAMP levels are controlled in mammary tissues and isolated epithelial and adipose cell preparations.

Progress: Epinephrine (EP) and prostaglandin E_1 PGE_1 treatments elevated cAMP levels in mammary tissue explants from virgin, pregnant and lactating mice whereas prolactin (PRL) was ineffective on the same explanted tissue. Freshly prepared adipose and epithelial cells as well as primary cultures of fibroblasts and epithelial cells derived from mouse virgin mammary gland were incubated with each of the agents. Each responded to EP and PGE_1 but only adipose cells responded to PRL. Mammary cells from mid-pregnant mice responded like those of virgin mice. Three of 15 tumor explants responded to PRL whereas 14 of 15 responded to PGE_1. No relationship exists between cAMP levels and tumor growth rate. Mammary cAMP levels decrease in mid-pregnancy, increase in late pregnancy and remain elevated during lactation. Addition of serum and insulin to explants incubated 4 hours in McCoys medium doubled the cAMP levels prior to DNA synthesis between 0 and 2, and 8 and 12 hours after addition to medium. Seven cell lines were established and karyotyped; 2 mammary tissue and 5 mammary tumors. Six lines are tumorigenic.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $80,000

CONTRACT RESEARCH SUMMARY

Title: Control of DNA Synthesis in the Mammary Gland

Principal Investigator: Frank E. Stockdale, M.D., Ph.D.
Name/Address Stanford University
Performing Stanford, California 94305
Organization:

Contract Number: N01-CB-23875
Starting Date: June 1, 1972 Expiration Date: May 31, 1976

Goal: To determine the role of serum factors and hormones in the initiation and control of DNA synthesis in mammary gland epithelium and mammary neoplasms of mice.

Approach: Studies include: 1) The isolation, characterization and source of factors in serum responsible for mammary epithelial growth; 2) Comparison of their mode of action with that of hormones which are also related to the growth of the gland; 3) Determination of the early cellular biochemical events which precede serum factor and hormonal initiation of DNA synthesis.

Progress: Mammary serum factor (MSF) exists in serum which initiated DNA synthesis in normal mouse mammary epithelium (MME) *in vitro*. The factor was purified 300-500 fold and the activity resides in 2 major protein fractions with molecular weights of 50-80,000 at neutral pH and in 3 minor proteins. MSF levels in serum are affected by hypophysectomy with a loss of 50% activity. Injection of growth hormone, prolactin, or estrogen does not restore activity. Prolactin (PRL) alone or in combination with serum does not promote growth of MME. High concentrations of PRL (5 to 20 ugm/ml) inhibit MSF initiated DNA synthesis but not insulin initiated DNA synthesis. This suggests MSF is under pituitary control but not growth hormone or PRL and that a receptor site for MSF may be similar or near the cell membrane PRL receptor site. Insulin initiated DNA synthesis in MME differs from that initiated by MSF. Either 2 cell types exist in the mammary gland or changing reactive sites exist for insulin and MSF initiated growth as the mammary gland develops. A monolayer culture of MME is used for bioassay which yields 4-600 assays per experiment. A monolayer of epithelium from mid-pregnant animals responds independently to insulin and MSF as in organ culture. Seven samples from breast cancer patients are tested routinely for MSF activity. Initiated studies on MTV-induced CFZ mammary tumor of Balb/c mouse which grows well as a monolayer culture. Monolayer cells are responsive to insulin but not to the same concentrations of serum that produces maximal growth responses in MME which suggests this tumor lacks MSF receptors or produces MSF-like factors that are unresponsive to MSF.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/10/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: Approx. $85,000

CONTRACT RESEARCH SUMMARY

Title: Neoplastic Potential of Preneoplastic Lesions of the Human Mammary Gland

Principal Investigators: S. R. Wellings, M.D.
Name/Address Hanne Jensen, M.D.
Performing University of California Medical School
Organization: Davis, California 95616

Contract Number: N01-CB-43908
Starting Date: June 28, 1974 Expiration Date: June 27, 1976
Goal: Define the growth potential of hyperplastic-dysplastic lesions of the human mammary gland also termed "preneoplastic" or "latent cancer cells,"

Approach: Analysis of the growth potential of human mammary heterotransplants in athymic mice. Transplants of ducts, lobules, carcinomas *in situ* and evident carcinomas will be implanted into female and male mice. The site of the implant will be cleared fat pad as well as intraperitoneally and subcutaneously. Also some transplants will be done with primary cultures following brief *in vitro* cultivation of the lesions. Vascular and inflammatory response of the host to the transplants will be determined and the difference between biopsy and autopsy material will be assessed. The hormonal milieu of the host will be manipulated in reference to growth capacity of transplants.

Progress: Twenty mastectomy and 26 biopsy specimens provided 292 transplants into nude-athymic mice. Most transplants were normal appearing lobules. Transplants 3-6 mm in diameter survived best. The cleared fat pad was the best site whereas the subcutaneous site was suboptimal and intraperitoneal site the poorest. Transplants into young female mice evoked a greater vascularization than those in older or in male mice. *In vitro* maintenance prior to transplantation caused some dedifferentiation. Six transplants of normal appearing lobules from 2 mastectomy specimens underwent dedifferentiation after 6 months *in vivo* of which 85% were in the cleared fat pad and the rest in the subcutaneous site. Of all transplants, 73% were in female and 27% in male mice; 80% were in the subcutaneous site, 8% in cleared fat pad and 12% in the intraperitoneal site. Sixty-nine percent of the transplants were found at autopsy of which 46% were vascularized and 43% were well preserved 3 weeks to 6 months after transplantation. Very few atypical lobules were found to transplant. More mastectomy specimens from women ages 40-50 will be examined which may yield more atypical lobular lesions.

Significance for Cancer Research (NCP Objective 4,5 Approach 2,4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group. Breast Cancer Steering Committee
FY'75 Funds: Approx. $175,000

CONTRACT RESEARCH SUMMARY

Title: An Evaluation of Surgical Adjuvant Chemotherapy in Patients with Breast Cancer

Principal Investigator: David L. Ahmann, M.D.
Name/Address Mayo Clinic
Performing Rochester, Minnesota 55901
Organization:

Contract Number: N01-CB-33899
Starting Date: June 30, 1973 Expiration Date: June 29, 1976

Goal: To assess the effects of surgical adjuvant chemotherapy, utilizing a multiple drug program in patients with locally advanced mammary carcinoma.

Approach: Patients with unfavorable primary breast cancers have been assigned to treatment programs involving mastectomy followed by chemotherapy, or followed by radiation therapy and chemotherapy. After stratification according to menstrual category, tumor size and degree of nodal involvement, patients are randomly assigned to one of three treatment groups. These are 1) l-phenylalanine mustard in five day courses every 6 weeks, 2) 5-fluorouracil, cyclophosphamide and prednisone on a similar schedule and 3) radiotherapy plus the 3-agent program. The adjuvant chemotherapy is initiated two weeks postoperatively, during radiotherapy when it also is given. Chemotherapy is terminated at the end of one year or upon the appearance of metastatic disease, whichever occurs first.

Progress: Patient entry into the study began in September 1973. Follow-up data are available upon all patients; no conclusions can as yet be drawn about the relative effectiveness of the above therapeutic regimens. Protocol revisions were made during FY75 after the report of the preliminary findings of the National Surgical Adjuvant Breast Project concerning the apparent advantage of postmastectomy chemotherapy. The protocol dropped the postmastectomy "observation only" and "radiotherapy only" arms and added the postmastectomy PAM regimen.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: Pending
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Studies in Predictive Transplantable Animal Mammary Tumor Models

Principal Investigator: Arthur E. Bogden, Ph.D.
Name/Address Performing Organization: Mason Research Institute
Worcester, Massachusetts 01608

Contract Number: NO1-CB-43914
Starting Date: November 1, 1965 Expiration Date: Oct. 31, 1975

Goal: To carry out preclinical evaluations of single and combined therapeutic modalities in experimental mammary tumors of known biological characteristics.

Approach: Studies are being done in four transplantable rat mammary tumor systems. The primary and metastasizing test-system, 13762 mammary adenocarcinoma, is used most extensively. The others, R3230AC, MT/W9a and R-35 mammary tumors are used to further test and evaluate those drug combinations and therapies which show promising activity in the 13762 system. The clinically active chemical anti-tumor agents of current interest include Adriamycin, Cyclophosphamide, 5-Fluorouracil, Prednisone, Vincristine, Dibromodulcitol, Methotrexate, Phenylalanine Mustard, and Methyl-CCNU. Chemotherapy assays of primary tumor grafts are made by initiating drug treatment on day 1, or day 15 when tumor is well-established. Metastasis assays of various treatment regimens are carried out by excising the tumor graft on day 18 and examining the animals for evidence of metastases at necropsy. Hormone radioimmunoassays are performed on selected animals with emphasis on the studies which determine the effects of drug treatments on the endocrine system. The effectiveness of immunological adjuvants (nonspecific immune response stimulators) is being explored in combinations with surgery, chemotherapy and X-irradiation.

Progress: Observations on the effects of various treatments include: 1) Phenylalanine mustard (PAM) is more effective and less toxic than cytoxan (CTX) but regrowth of crossresistant tumors occurs with either drug treatment. 2) Three-drug combinations of CTX with 5-fluorouracil (5-FU) and methotrexate (MTX) and of PAM with 5-FU and MTX were synergistic and produced long-term remissions and apparent cures. 3) The 2-drug combination of PAM and 5-FU was more effective than PAM and MTX. PAM was also effectively used in sequence with 5-FU. 4) The dose levels used in the above drug combinations produced minimal toxicity and permitted prolonged treatment and remission maintenance. 5) A portion of the regrowing PAM resistant tumors again become drug-responsive and regress under treatment during prolonged therapy. 6) The different mammary tumor systems show variations in chemotherapy responsiveness.

Significance for Cancer Research (NCP Objective 6C Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Treatment Site Visit Date: 3/22/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $126,300

CONTRACT RESEARCH SUMMARY

Title: Investigation of Steroid Sulfation and Estrogen-Binding in Human Breast Cancer

Principal Investigator: Thomas Dao, M.D.
Name/Address Performing Organization: Roswell Park Memorial Institute
Buffalo, New York 14203

Contract Number: N01-CB-43900
Starting Date: February 1, 1974 Expiration Date: Jan. 31, 1976

Goal: To determine the relationship of steroid sulfotransferase activity to estrogen receptor protein levels, to pathologic staging, and to risk of relapse.

Approach: Steroid sulfating enzyme activity and estrogen receptor (ER) levels will be assayed on breast tissue obtained from approximately 300 patients. Those women who develop clinical evidence of relapse will undergo appropriate endocrine therapy and their clinical responses will be documented. The evidence will be evaluated for or against correlation of the steroid sulfotransferase activity with risk for tumor metastasizing potential and with response of tumor to endocrine treatment. The predictive potentials of ER and sulfation activity will be compared. The investigators will continue their efforts to refine the biochemical techniques.

Progress: More than 150 patients have been entered into the study. About one third of these had lymph node metastases suitable for analysis as well as primary breast cancer. Of the tissues tested so far, almost 100% sulfated steroids. The ratio of dihydroepiandrosterone sulfate to estradiol sulfate (DHEAS/E_2S) was >1 in similar percentages of ER positive and ER negative tumors. Findings are also similar in tissue from patients with known tumor limited to the breast and from those with up to 3 positive axillary nodes. The data for >4 node metastases are too small at present to permit analysis.

Significance for Cancer Research (NCP Objective 6 Approach 6,4)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breat Cancer Treatment Site Visit Date: 6/5/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY/75 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Group (NSABP)

Principal Investigator: Bernard Fisher, M.D.
Name/Address University of Pittsburgh
Performing Pittsburgh, Pennsylvania 15261
Organization:

Contract Number: N01-CB-23876
Starting Date: June 30, 1972 Expiration Date: June 29, 1976
Goal: To determine the effectiveness of local treatments for Stage I breast cancer and of local plus systemic treatments for Stage II breast cancer.

Approach: The investigations are prospective and randomly assign a treatment program to each patient. Investigators from approximately thirty collaborating institutions participate in each protocol study. This assures rapid accession. Patients with clinical Stage I breast cancer are eligible to enter the study which tests the relative merits of two or more currently accepted forms of surgical and/or radiation treatments. Morbidity and cosmesis are considered as well as relapse rates and survival. In a separate clinical trial, patients with one or more positive axillary nodes receive l-phenylalanine mustard (PAM) or PAM plus 5-fluorouracil in an intermittent dosage schedule for two years or until evidence of treatment failure is documented, whichever comes first. The chemotherapy begins 2 to 4 weeks after mastectomy.

Progress: The NSABP investigators completed entry of patients into two large studies and reported their preliminary results at the September 30, 1974 "Report to the Profession" meeting. In 1800 patients who had undergone either radical mastectomy, or total (simple) mastectomy or total mastectomy plus radiotherapy, no significant differences had occurred in treatment failure rates or survival. These patients will be observed indefinitely to determine long-term effects. In the other completed study patients who had demonstrated tumor metastases in axillary nodes at the time of mastectomy received long-term intermittent PAM or placebo pills. The early tumor recurrence rate was lower in the premenopausal patients who received active drug and who had four or more positive nodes.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 12/14/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $500,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Charles Hubay, M.D.
Name/Address Case Western Reserve University
Performing Cleveland, Ohio 44106
Organization:

Contract Number: N01-CB-43990
Starting Date: June 16, 1974 Expiration Date: June 15, 1976
Goal: To test the effects of adding chemotherapy, anti-estrogen therapy, and BCG to mastectomy in patients with locally advanced breast cancer.

Approach: Patients who show axillary nodes involved with metastases at the time of surgery are eligible for this study. Stratification, but not treatment selection, is on the basis of the presence or absence of estrogen receptor protein (ER) in the tumor. Random treatment assignments are 1) observation only (control), 2) cyclophosphamide, methotrexate, 5-fluorouracil, tamoxifen (CMFT) for 6 months,or 3) CMFT for 6 months plus BCG for 6 months. Endpoint is the first evidence of treatment failure, i.e., the appearance of locally recurrent or of distant tumor.

Progress: Patient entry into the study began in September 1974. Accession is faster since the requirement was dropped that 4 or more of the axillary nodes show metastases. There have been no serious problems with toxicity due to treatment. No data are available as yet concerning treatment failure incidence.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 4/1/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $180,000

CONTRACT RESEARCH SUMMARY

Title: Prediction of Hormone Dependency in Human Breast Cancer

Principal Investigator: Elwood Jensen, Ph.D.
Name/Address University of Chicago
Performing Chicago, Illinois 60637
Organization:

Contract Number: N01-CB-43969
Starting Date: June 16, 1966 Expiration Date: June 15, 1976

Goal: To develop techniques which will increase the predictability of response of human breast cancers to endocrine therapy.

Approach: Since 1967 Dr. Jensen has been studying estrogen receptors (ER) and more recently has been examining the relationship between ER level in human breast cancer tissues and response to endocrine manipulative treatments. Patients who have had ER assays performed on their primary and/or metastatic tumors undergo additive or ablative endocrine procedures considered appropriate for their gonadal status, general medical condition, etc. In most instances the choice of therapy does not depend on the ER assay results. The data are analyzed for correlation between clinical response and ER levels. The investigators are also working on methodology for other receptor protein determinations and on modifications of the estrophile assay method, in particular a simple radioimmunoassay method.

Progress: 594 primary breast tumors have been studied and about 40% contained significant amounts of ER (>750 femtomoles/gram tissue in postmenopausal patients and >250 femtomoles in premenopausal). Of the patients with metastatic breast cancer, 131 underwent some type of endocrine therapy, the clinical results of which could be evaluated. 45 of these patients had ER positive tumors and 29 (64%) showed objective remissions. Only 2 of the 86 (3%) patients whose tumors showed less than significant amounts of ER had objective tumor remissions. Preliminary observations with 13 patients suggest that ER determinations in the primary tumor at the time of mastectomy may be used to predict tumor response to endocrine therapy if metastases appear in later years. In methodology, further assessment of the 4S complex is underway. The attempts to develop a simple radioimmunoassay for estrophilin are in their initial stages. Rabbits have been immunized with a highly purified preparation of the nuclear estradiol-receptor complex of calf uterus. As soon as sera are obtained that can be shown to contain antibodies, cross reactivity with the receptor of human breast cancer will be tested.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Ihor Masnyk, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 10/23/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $109,000

CONTRACT RESEARCH SUMMARY

Title: Study of Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Name/Address Performing Organization:	Charles Perlia, M.D. Rush-Presbyterian-St. Luke's Medical Center Chicago, Illinois 60612

Contract Number: N01-CB-43991

Starting Date: June 17, 1974 Expiration Date: June 16, 1976

Goal: To assess the effect of adding chemotherapy to oophorectomy for treatment of metastatic breast cancer.

Approach: Premenopausal patients undergo surgical castration upon demonstration of the first relapse following treatment of primary breast cancer. When evaluation is carried out 12 weeks after castration, antitumor effect is classified as failure, no change, or regression. The patients showing no change or tumor regression are randomly assigned to 1) observation or 2) treatment courses of cyclophosphamide, 5-fluorouracil, and methotrexate (CMF). When relapse is documented in the patients assigned to observation, CMF treatment will be started. This program provides comparison of the effectiveness of concomitant and sequential multimodal therapy.

Progress: The collaborating investigators began entry of patients into the study in August 1974. Insufficient time has passed to produce conclusive data about the effects of treatment.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.

Program: Breast Cancer Treatment Site Visit Date: Pending

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Steering Committee

FY'74 Funds: $123,300 (24 months)

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Joseph Post, M.D.
Name/Address Performing Organization: Goldwater Memorial Hospital, New York University
New York, New York 10017

Contract Number: N01-CB-33903
Starting Date: June 28, 1973 Expiration Date: June 27, 1976
Goal: To provide information concerning cell cycle parameters in patients with breast cancer.

Approach: *In vivo*: 1) ^{3}HTdR is given i.v. as a pulse label and serial biopsies are made to generate a labeled mitosis curve and to study grain count decay. 2) local lesions are injected with ^{3}HTdR and sampled. 3) ^{3}HTdR is given i.v. as continuous infusion over several days and the lesions are biopsied. These techniques permit the estimation of the population engaged in DNA synthesis, the cell cycle parameters, the size of the proliferating pool and the source of newly dividing cells. *In vitro*: Parallel studies are done on tissues from *in vivo* studies as well as from primary surgical specimens. Cells are cultured and kinetic data gathered as in designs 1) and 3) *in vivo*, as well as chromosome numbers. Samples are dissociated enzymatically, and their pulse and continuous labeling estimated with ^{3}HTdR along with DNA synthesis times using double labeling with ^{3}HTdR and ^{14}CTdR. These data will establish the proliferative patterns of human breast cancer cells and the evaluation of the effects of anti-cancer therapy before clinical detection of change in tumor size.

Progress: Studies *in vivo* from six patients show small proliferating pools of cells (less than 10%), replicating slowly in comparison to normal gut and bone marrow cells. These data explain in part the predilection for cytotoxicity of many drugs for replicating normal populations. Of particular interest is the relatively large proportion of the mitotic cells derived from a population with long G_2 residence. In culture, much larger proliferating pools of cancer cells turn over more quickly than *in vivo*. These data question the validity of extrapolating drug responses of the cultured cancer cells to those of the tumor cells of the cancer-bearing patient. An *in vitro* labeling method has been developed, using dissociated cancer cells. The data show similar small proliferating pools and long S times, as found *in vivo*. This labeling method shows promise as a monitoring technique for effects of therapy.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Stanley Shackney, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $110,000

CONTRACT RESEARCH SUMMARY

Title: Suppression of Endocrine Function by Systemic Agents as Treatment of Human Breast Cancer

Principal Investigator: Richard J. Santen, M.D.
Name/Address Performing Organization: The Milton S. Hershey Medical Center of the Pennsylvania State University Hershey, Pennsylvania 17033

Contract Number:
Starting Date: Expiration Date:

Goal: To produce suppression of adrenal function with aminoglutethimide and to compare the effects upon human breast cancer with those of adrenalectomy.

Approach: Female patients with inoperable, recurrent, or metastatic breast cancer are admitted to this study which is expected to establish the optimal technique for aminoglutethimide (AG) blockage of adrenal steroid synthesis and glucocorticoid suppression of the reflex rise in ACTH. The feasibility of suppressing ovarian function with AG and a progestin will be investigated. The influence of AG upon prolactin secretion will be studied *in vivo* and *in vitro*. The effects on human breast cancer of endocrine suppression by these noninvasive techniques will be compared with those of surgical ablation.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., E. Brad Thompson, M.D.
Program: Breast Cancer Treatment Site Visit Date: 3/7/75
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $200,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Edward F. Scanlon, M.D.
Name/Address Evanston Hospital
Performing 2650 Ridge Avenue
Organization: Evanston, Illinois 60201

Contract Number:
Starting Date: Expiration Date:

Goal: To determine the effects of adding chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

Approach: Patients who have metastatic axillary nodes or Stage III breast cancers are admitted to this study program. After stratification according to primary tumor size, number of positive nodes, and menstrual status, the participants are assigned to one of three treatment schedules, 1) intermittent l-phenylalanine mustard (PAM), 2) 5-fluorouracil, cyclophosphamide and prednisone (FCP) in intermittent courses, and 3) FCP plus BCG. Two of the treatment schedules are identical with the Mayo Clinic adjuvant study, and the data will be cumulative. Treatment courses will be given for one year or until there is evidence for relapse, whichever occurs first. Immunologic evaluation will be performed every three months. Survival and recurrence free intervals will be computed for each treatment group.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 8/29/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $180,000

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Lewis M. Schiffer, M.D.
Name/Address Allegheny General Hospital
Performing Pittsubrgh, Pennsylvania 15212
Organization:

Contract Number: NO1-CB-43899
Starting Date: February 1, 1974 Expiration Date: Jan. 31, 1976
Goal: To develop methods of rapidly determining cell kinetic parameters of individual human breast cancers so that this information may potentially be used in the study of the natural history and treatment of these tumors.

Approach: The project utilizes the spontaneous C3H mouse mammary tumor with which techniques for in vitro measurement of DNA synthesis time, growth fraction and cell cycle time are developed. All in vitro studies are controlled with in vivo studies. The growth fraction is determined by the PDP (primer available DNA polymerase) assay. Similar experiments on human mammary tissues are performed with the eventual goal being totally in vitro studies. It is planned to study tumors after their exposure to radiation or to cytotoxic drugs.

Progress: Individual cytokinetic results are now obtainable in 7 days in the mouse. In vitro studies correlate well with in vivo controls. Although the DNA synthesis time is relatively constant, the ^{3}HTdR and PDP labeling varies. Thus each tumor must be considered individually. Correlations with tumor weight, volume and doubling times are being performed. Human tumors are continually being accessed for in vitro studies and in vivo controls.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Richard Simon, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $138,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II and Stage III Carcinoma of the Breast

Principal Investigator: Frank C. Sparks, M.D.
Name/Address Performing Organization: University of California at Los Angeles
Los Angeles, California 90024

Contract Number: NO1-CB-43917
Starting Date: June 17, 1974 Expiration Date: June 16, 1976

Goal: To evaluate the effectiveness of adding chemotherapy and immunostimulation to mastectomy for patients with locally advanced cancer.

Approach: Patients with any number of positive axillary lymph nodes are eligible to enter this study, which randomly assigns one of the following treatment schedules, 1) cyclophosphamide, methotrexate, 5-fluorouracil (CMF) and BCG, or 2) CMF plus BCG plus tumor cell vaccine. The tumor vaccine is produced from irradiated cells. Treatment will be given intermittently for a period of two years or until there is evidence of tumor recurrence, whichever occurs first. The patients undergo skin tests and evaluation of immunologic parameters before treatment begins and at intervals throughout the study. The effects of live tumor cell vaccine is being tested in a group of women with metastatic breast cancer. If no evidence of local tumor proliferation occurs, the live vaccine will be considered for adjuvant use.

Progress: During the first contract year, approximately 40 patients have been admitted to the study. None has discontinued treatment and no patient has developed recurrence of cancer. One elderly patient experienced significant and prolonged myelosuppression which necsssitated chemotherapy dosage modification. Total alopecia has occurred in one patient. The patients have had no lessening of their reactivity to skin test antigens while receiving the combined immunochemotherapy. The decision to add tumor cell vaccine to the protocol was acted upon late in the year and no statement can be made about its effects. The entire study has been active too short a time to permit conclusions about delay of tumor recurrence by either treatment regimen.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 4/3/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $225,000

CONTRACT RESEARCH SUMMARY

Title: Study of Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Charles Vogel, M.D.
Name/Address Emory University School of Medicine
Performing Atlanta, Georgia 30303
Organization:

Contract Number: N01-CB-43916
Starting Date: June 1, 1974 Expiration Date: May 31, 1976
Goal: To determine whether the incidence and/or duration of anti-tumor effectiveness are increased by combining hormone therapy and chemotherapy.

Approach: Postmenopausal women with their first evidence of metastasis are assigned to one of two treatment regimens: estrogen or estrogen plus cyclophosphamide, adriamycin, and 5-fluorouracil. In those patients assigned to the first regimen (estrogens only), chemotherapy is started upon documentation of treatment failure. The patients are observed for objective evidence of tumor regression, duration of remission, and quality of performance status.

Progress: Accession of patients into the study has been slower than anticipated. The eligibility criteria exclude patients who have had any type of prior hormonal therapy for metastatic breast cancer. The investigators and project officers agree that this restriction is necessary to validate the results. The principal investigator is actively seeking a limited number of potential collaborating institutions.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: Pending
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $86,000

CONTRACT RESEARCH SUMMARY

Title: Estrophile Binding and Estrophile Proteins in Human Breast Cancer

Principal Investigator: Federico Welsch, M.D., Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-43867
Starting Date: October 16, 1973 Expiration Date: Oct. 15, 1975
Goal: To determine whether the levels and properties of estrophilic proteins are related to the responsiveness of cancer to endocrine treatment.

Approach: This is a type I contract in that the contractor carries out determinations of estrophilic receptor protein (ER) levels on 500 breast cancer samples per year. These tissue are submitted by collaborating clinicians whose protocols have been approved by the project officers. The ER assays are performed by both the sucrose gradient and the charcoal methods and methodology is under constant monitoring. The laboratory also performs steroid sulfurylation determinations on tumor samples of sufficient size. The project officers receive the reports of the ER assay results from the laboratory and are also provided with clinical data by the collaborating physicians. The combined data will be analyzed after accession of about 1000 tissues to determine the degree of correlation between ER levels and tumor response to treatment.

Progress: The contractor has reached the capacity of 500 assays per year and applications have exceeded that number. The new protocols are being evaluated for relevance to the program. If it appears that more than 500 suitable tissues are available, additional support will be sought.

Significance for Cancer Research (NCP Objective 5B Approach 6)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Committee Site Visit Date: 6/7/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $70,700

CONTRACT RESEARCH SUMMARY

Title: Prolactin Radioimmunoassays in Human Serum

Principal Investigator: W. P. VanderLaan, M.D.
Name/Address Performing Organization: Scripps Clinic and Research Foundation
La Jolla, California 92037

Contract Number: NO1-CB-33881
Starting Date: June 29, 1973 Expiration Date: March 28, 1976
Goal: To perform radioimmunoassays of prolactin in serum from women who have breast cancer.

Approach: The contractor raises antibody in rabbits using 5 mg human prolactin (hPRL). The goal is an antiserum reacting only with hPRL of the human anterior pituitary hormones of a titer that can be used at a dilution of 1:5000 or higher. The antiserum is used to perform human prolactin radioimmunoassays for collaborating investigators, whose research protocols have been approved by the project officers and representatives of the Breast Cancer Treatment Committee. Results of the assays are available 2 to 4 weeks after the sera are received. The capacity is up to 1000 samples per month. The prolactin assay data are surveyed for relationship to clinical findings in patients with breast cancer. Methodology has been improved.

Progress: Problems with radioiodination of hPRL were shown to be due to storage techniques. Storage in the dry state appears to be advantageous. Increased speed in obtaining assay results has been made possible by reducing the incubation period before addition of the second antibody from three days to overnight. Collaborative studies have included thyrotropin releasing effect on prolactin concentrations, effects of fasting and of reduced physical activity, prolactin release patterns during lactation, and prolactin values from a large group of women who underwent mammography and who had supplied personal and family cancer histories. Another area of study involves the binding of fluorescent labeled prolactin to mammary tissue receptors. There have been fewer than expected protocols submitted which directly involve treatment of patients for breast cancer.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 4/2/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $82,500

CONTRACT RESEARCH SUMMARY

Title: New Techniques in Cell Kinetics of Breast Cancer

Principal Investigator: Robert M. Zucker, Ph.D.
Name/Address Performing Organization: Papanicolaou Cancer Research Institute
Miami, Florida 33123

Contract Number: N01-CB-33861
Starting Date: June 27, 1973 Expiration Date: February 26, 1976

Goal: Develop an approach to cell kinetics which is potentially adaptable to the study of human breast cancer kinetics and the cellular effects of chemotherapy.

Approach: The project utilizes a series of different rat and mouse mammary tumors to acquire information on the procedures necessary to obtain single cell suspension from solid tumors. A combination of mechanical and enzymatic procedures are used to yield single cells. These dissociated cells are to be used in velocity sedimentation and density gradients cell separation experiments. The physically separated cells and enzymatically dissociated cells will be characterized by cell cycle times, labeling indices, growth fractions, electron microscopy and histochemical tests. Chemotherapeutic drugs will be administered to tumor bearing animals and the specific cellular effect will be measured on the dissociated and separated cells. The dissociation procedures developed on animals will be applied to human breast tumors.

Progress: The investigations on four animal tumors (RAT 13762, Rat 1330AC, mouse mammary C3H and B16 mouse melanoma) demonstrated that the combinations of enzymatic and mechanical procedures are superior to either one alone. A combination of collegenase, trypsin, and mechanical agitation in a Ca^{++} free tissue culture medium gave high cell yields with viabilities exceeding 80% in the four tumors. The medium was supplemented with 2% bovine serum albumin (BSA) for cell protection and DNAse to inhibit cell clumping. Viable cells have also been enriched from dissociated cells by BSA and Ficoll-Hypaque step gradients. Using dissociated cells, preliminary results correlated the in vivo labeling index with the in vitro labeling index. Prior to autoradiography the cells were concentrated onto a slide in 10% BSA with the Shandon Cytospin. This technique preserves the cell morphology and facilitates rapid autoradiography by flattening the cells. Using dissociated cells, the cell separation procedures have demonstrated that velocity sedimentation is superior to buoyant density in separating S phase cells. Estradiol sensitive cells dissociated from DMBA induced rat tumors were identified by autoradiographic nuclear label. In contrast, the estradiol insensitive R3230AC tumor cells showed only cytoplasmic label.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Richard Simon, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY'75 Funds: $110,000

ANNUAL REPORT SUMMARY

CANCER DIAGNOSIS CONTRACT PROGRAM
July 1, 1974 to June 30, 1975

The Diagnosis Branch of the Division of Cancer Biology and Diagnosis has expanded its contract research effort rapidly since the Branch was reactivated in 1972. Its current research program, which is operated entirely by contracts, encompasses the categories listed below in the table. Expert advice for the program is provided by the advisory committees as indicated.

Research Categories	Advisory Committees
General Cancer Diagnosis	Diagnostic Research Advisory Group
Breast Cancer Demonstration Projects	Breast Cancer Diagnosis Committee
Breast Cancer Diagnosis	
Diagnostic Radiology	Diagnostic Radiology Committee
Cytology Automation	Committee on Cytology Automation
Immunodiagnosis	Committee on Cancer Immunodiagnosis

The program for Breast Cancer Diagnosis and Immunodiagnosis are presented in the other sections of this Annual Report; the other categories are described below.

GENERAL CANCER DIAGNOSIS STUDIES

Lung Cancer received early attention in the Diagnosis program. A joint study currently is in progress by a Cooperative Early Lung Cancer Group. In this study each of three projects (at Mayo Foundation, Johns Hopkins University Medical School and Memorial Hospital-Strang Clinic in New York City) will screen at least 5,000 persons who are heavy smokers annually for lung cancer with frequent sputum cytology and chest x-ray examinations. In each project 5,000 other screenees (of equal risk) will be randomly selected to act as a control cohort. Localization of lesions is accomplished with fiberoptic bronchoscopy. The goal is to detect lung cancer at a sufficiently early stage to permit its removal and cure by a surgical procedure that will be less extensive than a pneumonectomy. The University of Cincinnati Statistical Center will coordinate the accumulation of data from the three cooperating projects. A Manual of Procedures has been compiled by the group. Seven thousand five hundred persons have already been screened at the Mayo Clinic, approximately 2,100 persons at the Johns Hopkins Medical School and 1,500 at Memorial Hospital. At Johns Hopkins University Medical School studies continue on cytogenetics of lung cancer cells in sputum, immunological procedures in the detection of lung cancer and other procedures; studies on the use of tantalum powder in the localization of lung tumors are being completed. The Mayo Clinic is studying the role of aryl hydrocarbon hydroxylase enzyme in heavy smokers and persons with lung cancer. At Memorial Hospital in New York City, studies toward the development of a squamous cell antigen related to lung cancer will be undertaken. Work on a tantalum delivery system for localization of lung tumors has been completed at the University of Pittsburgh, and clinical and other studies of tantalum bronchography will go on at Johns Hopkins University.

Three cooperating institutions, Mayo Foundation, the University of Chicago and Memorial Hospital-Sloan Kettering Institute, are studying, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies are being made of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts. Such secretions are being studied for tumor-associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer. Other diagnostic methods are being investigated.

Several ongoing projects are concerned with various aspects of diagnosis of gastrointestinal cancer. Studies at University of Rochester and American College of Radiology are exploring methods of bowel preparation prior to barium enemas or colonoscopy. At the Mayo Clinic, there is a study of carcinoembryonic antigen in conjunction with other tests for cancer to determine if any of these combinations will assist in the earlier diagnosis of gastrointestinal cancer. Two projects, at Mt. Sinai Medical School and the University of Louisville, are investigating development of a quick, sensitive method for the detection of human blood in stool specimens; if such a method is forthcoming it will be applied to humans in a screening program to be developed.

Two projects, one at Stanford University and one a joint effort at SUNY-Buffalo and Roswell Park Memorial Institute, are studying biochemical markers which may be of help in specific or in general cancer diagnosis. Hormone markers for the same purpose are being studied at Harbor General Hospital.

Three projects, at Baylor University College of Medicine, Downstate Medical Center-SUNY and at Johns Hopkins University, are studying nuclear magnetic resonance in normal, non-neoplastic diseased tissue and neoplastic tissue. Certain physical signals called spin lattice (T_1) and spin-spin (T_2) relaxation times have been stated to be different in cancer tissue as compared to normal tissue. These contracts provide encouragement of more study in this area and will determine whether NMR signals from cancer tissue are specific or whether they result from granulomatus disease, necrosis or hemorrhage. The properly blinded studies, assuring objectivity, should establish the validity of this technique.

One project to evaluate the "Xonics" process for electron radiography is being carried out at Hahnemann Medical College.

In addition to the above twenty-one projects in General Cancer Diagnosis, five others are pending. All have been approved, awards have been recommended and contracts currently are being negotiated.

BREAST CANCER DEMONSTRATION PROJECTS

Much of the initial thrust of the Contract Diagnosis program was directed toward research in earlier detection of breast cancer; this has culminated in the establishment of 27 regional Breast Cancer Demonstration Projects. Twenty-seven of these projects currently are in operation and by spring 1975 approximately 150,000 women had been screened. None of the projects have experienced any serious problems in recruiting which has been carried out in an excellent manner by local American Cancer Society units. Most of the projects have waiting lists of at least six months. From the approximately one-dozen projects site-visited during the past few months, it appears that response to annual recalls may be approximately 90 percent. Recall mechanisms are under constant review. Also under review with each site visit were the methods of physical examination and the teaching of breast self-examination and the methods for mammography and thermography reading. Constant stress is placed on significance of independent interpretation of each of the three diagnostic procedures.

One meeting has been held with the Project Pathologists to define their role in formalizing the information obtained from biopsy and other surgical specimens. Fundamentally, the Project Pathologist is responsible for completing the pathology forms which have been revised and distributed to all projects. The surgical material will be reviewed locally, regionally and centrally; the latter two reviews may be by sampling. From this effort it is expected that attention will be directed to the pathology of early breast cancers and possibly to its precursors.

In late February, a meeting was held of Project Directors and in conjunction with this meeting a thermography workshop was conducted. The review was under the guidance of Dr. JoAnn Haberman's Committee on Thermography Quality Control.

The Data Management Center for collection and statistical treatment of all data collected by the 27 projects has been established at the University City Science Center in Philadelphia. Standard reporting procedures have been developed. Preliminary analysis of data from approximately 90,000 women entered into the computer as of the end of 1974 indicates that biopsies have been recommended for approximately 4 percent of those examined. At least one-half of those recommended for biopsy have been performed. Twenty-two percent of those biopsied have been reported as cancer. These fragmentary results, on a small population sample, are regarded as quite preliminary and should not be used as a basis for any conclusions.

DIAGNOSTIC RADIOLOGY

In this area of research the improvement of x-ray imaging has been a matter of major concern and one to which the Diagnostic Radiology Committee has devoted much time. For early cancer detection and for screening programs involving periodic recurrent x-rays of individuals, it is extremely important to achieve improved resolution with substantially lower levels of radiation. A number of new imaging methods have been considered but most appeared to have insufficient promise to warrant support at the present time. One project at Hahnemann Medical School, to evaluate a new method of x-ray imaging developed by Xonics Corporation, shows promise. Five institutions: Massachusetts General Hospital, Columbia Presbyterian Medical Center, the Mayo Clinic, George Washington University Medical School and the Cornell University Medical School, have projects to evaluate new equipment for computer assisted tomography of the brain, a noninvasive technique believed to be of great promise in the field of neuroradiologic diagnoses. It is anticipated that this new technique will eventually replace present techniques used in diagnosis of intracranial lesions. Projects to investigate the use of proton beams in tissue density measurement are in progress at the University of California and University of Chicago.

In addition to these ongoing projects, contracts are currently being negotiated for several others in the general area of diagnostic radiology and work will begin within the next few months. These include several projects related to computerized transaxial reconstruction and are concerned with equipment development for computerized body transaxial x-ray reconstruction; development of algorithms for x-ray and nuclide reconstruction; and construction of projection data bases to test such algorithms.

CONTRACT RESEARCH SUMMARY

Title: Development and Testing of a System to Improve X-Ray Imaging, Contrast and Sharpness by Elimination of Scatter, While Retaining the Resolution of Film

Principal Investigator: Name/Address Performing Organization:	Gary T. Barnes, Ph.D. University of Alabama Birmingham, Alabama 35203

Contract Number: N01-CB-53865

Starting Date: Expiration Date:

Goal: To remove scatter and thus improve sharpness and contrast which will in turn improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.

Approach: To construct a system that will function in a clinically useful range of exposure times and delivered dose and that can obtain the stated primary goals by mechanical and/or electronic means and should eliminate the need for grids and air gaps. Methods will be developed to determine the quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast and dose reduction. Feasibility should be established during the first year of this two-year contract.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer:

Program: Diagnosis Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator:	Arthur J. Present, M.D.
Name/Address	American College of Radiology
Performing	20 N. Wacker Drive
Organization:	Chicago, Illinois 60606

Contract Number: N01-CB-43974
Starting Date: June 15, 1974   Expiration Date: June 14, 1975

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time (within a 24-hour period) and without excessive dietary restrictions and without excessive patient discomfort or inconvenience.

Approach: Under the administrative supervision of the American College of Radiology, the comparative efficiency of eight selected colon cleansing regimes will be studies by eleven institutions. A total of 6600 patients (600 patients in each institution) will be examined; all regimes will be employed by each of the participating institutions on a randomized basis, standard forms for the collection of pertinent clinical information and for the evaluation of the efficiency of each regime have been prepared and will be completed at each institution. Methods for cross evaluations are also included. Statistical analyses will be performed by the Department of Biomathematics at M.D. Anderson Hospital.

Progress: As tentative approaches to patient evaluation began, the need to revise protocols became evident. This was due to the strict contraindications for patient selection. Protocols limiting study patients and not affecting the scientific validity of the study were revised. Additionally, the sample size per protocol was reduced from 50 to 25 patients with intent of performing the study in two complete series. This allows for a complete sample with limited data after approximately one year. With the aforementioned revisions, studies are under way with participating institutions ranging from the first to the third series of assigned protocols.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis   Site Visit Date: March 19, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $173,155

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues

Principal Investigator: Carlton F. Hazlewood, Ph.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas 77025
Organization:

Contract Number: N01-CB-43978
Starting Date: June 30, 1974 Expiration Date: June 29, 1976

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.

Approach: To determine T_1, T_2 and T_1/T_2 in breast lesions. To conduct a blind study on surgical specimens in conjunction with Department of Pathology. To explore the relaxation time in the rotating frame (T,p), compound relaxation times, temperature dependence of relaxation times, feasibility of using relaxation time in detection of cancer surrounded by normal tissue.

Progress: Using NMR techniques to measure relaxation times of water hydrogens, it has been demonstrated that specific morphological states of various tissues can be recognized. In a study of well-defined human breast biopsy material, both relaxation times T_1 and T_2 of adenocarcinoma samples could be distinguished from normal and fibrocystic tissue. In addition, the T_1/T_2 ratio made it possible to distinguish fibroadenomas from adenocarcinomas.

During the past six months two major problem areas have been defined which when solved will permit a better assessment of the overall value of NMR in breast cancer detection and diagnosis. (1) Breast biopsy samples received are heterogeneous and often contain little diseased tissue, and (2) this tissue produces multiple relaxation times. These relaxation times can be "dissected" and identified with tissue types (i.e., cancerous cells produce longer and normal cells shorter relaxation times). Once this problem is delineated, specifications for low-cost miniature spectrometers can be established. Premalignant tissue can be distinguished in animal models from both normal and malignant tissue and human "non-cancerous" tissues are being screened to see if high risk populations might be defined. Completion of the analysis of approximately 500 breast samples is expected by the end of May, 1975.

Significance for Cancer Research (NCP Objective 5 Approach 5)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: February 6, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $76,458

CONTRACT RESEARCH SUMMARY

Title: Determination of Optimal Frequency of Screening Strategies

Principal Investigator: Name/Address Performing Organization:	Arthur Albert, Ph.D. Boston University Boston, Massachusetts 02215

Contract Number: N01-CB-53863
Starting Date: Expiration Date:

Goal: To develop a means whereby the optimum frequency of screening can be determined for early cancer for various anatomic sites in order to assure success in such screening as the least possible cost and hazard to the patient and still be effective in reducing death from cancer.

Approach: A team of clinicians, pathologists, statisticians, operations researchers and epidemiologists will carry out extensive investigation and documentation of the level of present knowledge, both empirical and theoretical on the following five factors: 1) the natural history or development of the disease, 2) the incidence of the disease in the target population, 3) the effectiveness (sensitivity and specificity) of the various available screening tests at each stage of the disease, 4) the effectiveness (on mortality) of intervention (treatment during each stage of the disease) and 5) the cost and hazards of the various screening tests. Disease models will be developed and validated for which screening data exists, or must be developed, such as colon, lung, bladder and prostatic cancer. Through parametric analysis these models will be used to suggest general ranges or minimum limits on the performance of screening tests if they are to be effective in early cancer detection and reducing cancer mortality.

Progress: New Contract

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer:
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis

Principal Investigator: Name/Address Performing Organization:	A. R. Moossa, M.D. University of Chicago Chicago, Illinois 60637

Contract Number: NO1-CB-43976
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the five-year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor-associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: Twenty-two of the 41 total patients have been fully investigated and 10 have undergone laparotomy. Indication for surgery in the remaining 12 patients decreased as investigations proceeded. Of the remaining 19, 2 were diagnosed elsewhere as having pancreatic carcinoma, and blood was taken from them for antigen and hormone assays. The other 17 patients were partially investigated; their study was discontinued at their physicians' request. In this group 8 laparotomies were performed. Eighteen laparotomies have yielded 6 pancreatic carcinomas, 2 islet tumors, 1 retroperitoneal lymphoma, and 1 carcinoma of the duodenum. In addition, 1 patient had widespread carcinoma for which no primary was discovered, 4 patients were found to have chronic pancreatitis, and the remaining 3 laparotomies were negative.

Significance for Cancer Research (NCP Objective 5 Approach 3)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: January 27, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $174,594

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: Name/Address Performing Organization:	C. Ralph Buncher, Sc.D. University of Cincinnati Medical Center Cincinnati, Ohio 45219

Contract Number: N01-CB-43868
Starting Date: June 25, 1974 Expiration Date: May 31, 1979

Goal: To collect and analyze data from the three collaborating lung cancer groups in the lung cancer detection program directed to the detection of early lung cancer in high risk patients to see if this will reduce mortality and morbidity.

Approach: Procedure manual will be prepared and forms will be created so that uniform data will be received by the Statistical Center. The data will be monitored, entered into the computer and analyzed. Reports will be made quarterly. Statistics will be analyzed for significance.

Progress: During the first twelve months of the contract, the Cincinnati Central Statistical Group (CSG) has contributed to the success of the Early Lung Cancer Diagnosis Project by identifying a common data base and making suggestions for uniform data recording and reporting to the NCI and participating clinical centers. The CSG has received patient data from each of the clinical centers and formed a master patient file from which tables, reports and analyses can be derived to answer the questions of the study; i.e., whether efforts directed toward earlier diagnosis and improvement of mortality have been successful.

Tables showing the results and status at each of the clinics have been available for the majority of the year and have proven of value to the NCI and other participants.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: December 18, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $175,744

CONTRACT RESEARCH SUMMARY

Title: Clinical Application and Evaluation of "Xonics" Electron Radiography

Principal Investigator: Name/Address Performing Organization:	Luther W. Brady, M.D. and Leonard Stanton, M.S. Hahnemann Medical College and Hospital Philadelphia, Pennsylvania 19102

Contract Number: N01-CB-43992
Starting Date: June 30, 1974 Expiration Date: June 29, 1976

Goal: An evaluation of both present and improved versions of the Xonics X-ray imaging system, in order to report the following information: 1) Basic scientific information for technique design and optimization; 2) Extensive clinical experience to determine the most promising use of electron radiography, as well as studies where conventional radiography may be preferable; and 3) New approaches to the basic problem of comparing the ability of different imaging systems to demonstrate marginally detectable pathology.

Approach: In order to accomplish these objectives, the contractor will make basic scientific measurements as to response to X-rays, detail resolution, noise and reliability and reproducibility of clinical equipment. Finally, a clinical comparison and evaluation will be performed involving 16 nationally recognized diagnostic radiologists who are very interested in contributing to the evaluation process. This will include normals (40%) as well as films demonstrating pathology; pairs will not be presented as pairs, nor will each evaluator necessarily receive pairs.

Progress: The equipment now produces ERG's with good speed and quality. Dry unit is yielding wide latitude, edge enhanced images highlighting vascular and calcific detail. Little difference was found among six toners. Patient dose is higher for best ERG images, vs. conventional technique. Liquid unit is now operational. Its positive aspects: Dose is much lower vs. conventional technique, with sharp, controlled edge enhanced images of low granularity. Its negative aspects: Artefactual image smear mars detail resolution at present. Improvements are to be tested shortly. Novel edge sharpness and enhancement measurement techniques have been developed. Clinical ERG-conventional comparisons are being accumulated using both ERG development methods in preparation for radiologist panel studies. Acquisition of the new chest and mammography ERG units for evaluation is anticipated.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg and R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: January 22, 1974
Technical Review Group: Diagnostic Research Advisory Group March 26, 1975
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $79,320

CONTRACT RESEARCH SUMMARY

Title: Hormone Markers for the Detection and Diagnosis of Cancer

Principal Investigator: Name/Address Performing Organization:	William D. Odell, M.D., Ph.D. Harbor General Hospital Torrance, Los Angeles, California 90502

Contract Number: N01-CB-43903
Starting Date: June 1, 1974 Expiration Date: May 31, 1977

Goal: To develop hormone markers that might indicate the presence of small (early) treatable cancer.

Approach: 1) To use techniques and assays developed to measure ADH, vasotocin, oxytocin, alpha and beta MSH, ACTH, alpha and beta TSH, and alpha and beta HCG to search for tumor elaboration of these peptides among cancer patients; 2) To develop new techniques for separating and easily identifying "big forms" or "prohormone" forms of ACTH, gastrin, and pancreozymin-cholecystokinin (PZ-CCK); 3) To search for the existence of such big forms of: ADH, oxytocin, alpha and beta-MSH, luteinizing hormone, and alpha and beta-glycopeptides, 4) To develop peptide assays for fetal hormones related to vasopressin, such as mesotocin as well as other fetal peptides and proteins such as fetuin, and to search for their elaboration by cancers; and 5) To use the insights and techniques gained in these studies to identify other nonhormonal peptides which might be used as cancer markers.

Progress: Assay procedures for several hormones have been accomodated to large scale evaluation. Most of these assays have been applied to a well-selected control population of men over fifty, post-menopausal women, men and women under the age of fifty. Data for each of these assays have been compared with a select group of blood specimens from sixty cancer patients and of tumor extracts from nine neoplasms. A "normal" distribution of some of the peptide hormones does not exist, therefore special investigation is underway on the patients whose hormone measurements were in the high range. Samples from cancer patients were collected from the Mayo Clinic and from Harbor Hospital. Additional data on histology of tumor type, age, sex, and other data concerning medical status is necessary for full evaluation.
Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: February 28, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $159,712

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: Name/Address Performing Organization:	John K. Frost, M.D. Johns Hopkins University Baltimore, Maryland 21218

Contract Number: N01-CN-45037
Starting Date: September 1, 1973 Expiration Date: August 31, 1975

Goal: To determine the effect of the early detection of lung cancer by sputum cytology followed by surgical removal on long-term survival in the disease.

Approach: The study population will be males, 45 years or older, who smoke a pack or more of cigarettes a day. By random assignment from individuals responding to advertisement, it is planned to accumulate over several years two groups of 4,000 each. The test group will receive sputum cytologic examination and chest X-ray every four months; the control group will have an annual chest X-ray, present good medical practice. Subjects suspicious or positive for cancer will be carefully worked up, including fiberoptic bronchoscopy, and cancers will be removed surgically. All subjects will be followed for five years to determine actuarial survival. (See also N01-CB-92172).

Progress: There have been 2100 people screened to date in this program. A return rate of 3 percent was obtained from miscellaneous group; while a return rate of 5 percent was obtained from the others. Screening was done at Johns Hopkins (downtown Baltimore) and Social Security (Baltimore beltway). Cancers found to date at approximately 3 per 1,000 screenees, about equally divided in both the control X-group and the cytology C-group. This project is carried on in conjunction with Mayo, Memorial, and Cincinnati in the NCI Cooperative Early Lung Cancer Group.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early cytologic detection, the best present hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: June 1973; February 3, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $630,811

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer - Early Detection, Localization, and Therapy

Principal Investigator: Name/Address Performing Organization:	John K. Frost, M.D. Johns Hopkins University Baltimore, Maryland 21218

Contract Number: NO1-CB-92172
Starting Date: June 28, 1969 Expiration Date: June 27, 1975

Goal: 1) Develop improved cytologic methods for sputum examination for the early detection of lung cancer; 2) Develop improved methods for the diagnostic localization of lung cancer.

Approach: The project follows a group of chemical and asbestos workers at high risk of lung cancer for sputum cytology studies. These are directed to the biologic significance of the morphology of mild, moderate and severe cell atypia, by correlation with patient follow-up. Patients cytologically suspicious or positive for cancer are studied for better localization techniques, mainly with tantalum dust bronchography and fiberoptic bronchoscopy. Improved equipment is also under development. (See also NO1-CN-45037)

Progress: Clinical material was obtained from 2,165 cigarette smokers, asbestos and bis-CMME workers. Developed improved transbronchial television microscope and new methods to diagnose cancer cytologically and histologically from areas of lung heretofore unobtainable. For x-ray negative lesion localization, developed controllable catheter tantalum bronchography and various instruments for rigid and flexible fiberoptic bronchoscopy. Surgical techniques developed for improved therapy of early lesions. Techniques evolved from this Phase I are used in J. H. Phase II, and by Mayo and Memorial in Early Lung Cancer Cooperative Study. Continuing: Investigation of the biologic significance of cytologic high-suspect lesions; parameters for automatic recognition of lung cancer; development of transbronchoscope probes for mucosal tension, bronchial wall firmness, infrared sensing, thermorecovery, laser, electrocautery, and ultrasonic forceps; development of clinical inhalation for tantalum bronchography.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early detection is the best present hope for improving the mortality from lung cancer.

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: May 7, 1974;
Technical Review Group: Diagnostic Research Advisory Group February 3,
Relevance Review Group: Cancer Diagnosis Steering Committee 1975
FY 74 Funds: $488,527

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues

Principal Investigator: Name/Address Performing Organization:	Donald P. Hollis, Ph.D. Johns Hopkins University Baltimore, Maryland 21218

Contract Number: N01-CB-43911

Starting Date: June 1, 1974 Expiration Date: May 31, 1976

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.

Approach: To carry out measurements of T_1 T_2 and T_{1p} on a variety of malignant, normal and diseased but not malignant tissues removed at surgery; these will be carried out blindly in conjunction with the Department of Pathology. There will also be an evaluation of the sensitivity of NMR measurement to small quantities of malignant cells mixed with normal cells.

Progress: Thus far, primarily T_1 measurements have been made. To date, 279 specimens of human tissue, removed from 118 separate patients in the Johns Hopkins Hospital, have been examined. These specimens included normal tissue, benign neoplastic tissues and various kinds of cancers. Based on data gathered to date it is concluded that NMR cannot be used in this direct manner for diagnosis of human cancer because it is not specific for cancer cells. Water content of the tissue is the main determining factor in fixing the value of the T_1 relaxation time. It still is possible that NMR may have a valuable role in cancer detection in the sense of screening from a population those who have a higher than normal likelihood of bearing malignancy. During the remainder of this project year and the second year of the project, emphasis will be shifted from measuring T_1 measurements by the null method to the use of the method of partially relaxed spectra in order to insure that we are observing only water protons.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: Louis P. Greenberg

Program: Diagnosis Site Visit Date: February 4, 197

Technical Review Group: Diagnostic Research Advisory Committee

Relevance Review Group: Diagnosis Steering Committee

FY 74 Funds: $60,701

CONTRACT RESEARCH SUMMARY

Title: Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or More (over 7-10 mls per 24 hours) of Human Blood in Human Feces

Principal Investigator: Name/Address Performing Organization:	Curtis L. Songster, M.D. University of Louisville Foundation, Inc. Louisville, Kentucky 40208

Contract Number: N01-CB-43968
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 mls in 24 hours) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor shall develop a procedure for the detection of human blood in feces by utilizing antigens specific for the globin portion (more specifically, Alpha chain) of hemoglobin. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry and fish diets and be truly negative by the proposed test.

Progress: High titer goat antisera have been raised against Alpha-chain of human hemoglobin. Cow, pig, sheep, chicken and fish hemoglobins lack cross-reaction. Cross-reactions with human hemoglobins are complete for A/A, A/S, A/C, S/S, S/C and monkey; partial with rabbit hemoglobin. Development of a latex agglutination test and complement-fixation assay for human hemoglobin is underway. A model utilizing goat IgG-coated latex and rabbit anti-goat serum has been optimized for experimental variables, including bead preparation, working and final latex concentrations, pH, ionic species and strengths. Hemoglobin has been demonstrated to bind to antibody-coated latex, but agglutination does not occur. An attempt is being made to facilitate agglutination by several methods. Human hemoglobin has been specifically detected in fecal samples from autoingestion and patients with upper G.I. bleeding.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: December 17, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $38,887

CONTRACT RESEARCH SUMMARY

Title: Detection and Localization of Early Lung Cancer

Principal Investigator: Name/Address Performing Organization:	Robert S. Fontana, M.D. Mayo Foundation Rochester, Minnesota 55901

Contract Number: N01-CB-53886 (Successor to Contract NOI-CB-22000)
Starting Date: September 27, 1974 Expiration Date: September 26, 1978

Goal: 1) Improve methods for the early diagnosis of lung cancer. 2) Assess survival of patients with lung cancer detected by these methods.

Approach: A population of high risk patients (i.e., heavy cigarette smokers) who come to Mayo Clinic for conditions other than lung cancer will be examined and randomized into two groups. A close surveillance group will receive chest x-ray examinations and sputum cytology tests three times a year. A standard surveillance group will have chest x-rays and sputum cytology examinations when first seen and will then be advised to have follow-up chest x-rays and sputum tests annually. It is hoped that all patients suspected of lung cancer either by cytology or by x-ray will return to the Mayo Clinic for treatment. Those with sputum positive for cancer cells, but with negative chest x-rays will be admitted for localization studies, including fiberoptic bronchoscopy. Localization of radiographically occult cancer will be followed by appropriate treatment, usually conservative resection. Computer analysis of chest x-rays and hematoporphyrin as an endobronchial tumor "marker" are also being studied.

Progress: There were 7,027 subjects enrolled as of January 1, 1975, including 3,350 during calendar year 1974. Initial screening has detected previously unsuspected cancer of the respiratory tract at a rate of approximately 8 per 1,000 screened. The "incidence" rate of new cancer detected following negative initial screening is currently 4.5 per 1,000 person-years surveillance. Forty cases of respiratory tract cancer were localized and treated during 1974, of which eleven were radiographically occult. Since initiation of the study a total of 84 cases have been localized and 32 of these have been occult. Approximately 2/3 of the 84 cases have been resected, including 11 of the 14 "incidence" cases detected during rescreening of those whose initial screening was negative. The prognosis appears to be good in 36 of the 84 total cases and in 9 of the 14 "incidence" cases.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis Site Visit Date: January 13, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $708,627

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen (CEA) Test, A New Method and Its Evaluation

Principal Investigator: Name/Address Performing Organization:	Vay Liang W. Go, M.D. Mayo Clinic Rochester, Minnesota 55901

Contract Number: N01-CB-23854
Starting Date: June 15, 1974 Expiration Date: June 14, 1975

Goal: a) To evaluate the carcinoembryonic antigen test in diagnosis of carcinoma of the large bowel; b) To continue evaluation of CEA as a biologic marker in patients undergoing chemotherapy, radiotherapy and immunotherapy of advanced gastrointestinal cancer and in patients following resection of gastrointestinal cancer with curative intent; c) To determine the value of simultaneous measurement of several fetal antigens in the same patient in the diagnosis of cancer; d) To continue to collect colonic malignant tissue for different investigators in CEA; e) To characterize the CEA-like material secreted into the human gastrointestinal secretion; f) To determine CEA level of all blood samples in the Tumor Plasma Bank located at NCI-Mayo Clinic facilities; and g) Evaluation of other new diagnostic procedures for diagnosis of large bowel cancer including colon cytology and Hemoccult test.

Approach: To date, 6,700 patients have been studied; 1,466 were cancer patients and 5,032 were noncancer patients. Of the 1,466 cancer patients, there were 757 patients with GI malignancy and 709 patients with no GI malignancy. Of the 5,032 noncancer patients, 3,398 were patients entered into the Mayo Lung Project.

Progress: Results of Prospective Evaluation of Serum CEA - A. In Health: 1) Serum CEA is not influenced by sex, age, blood type, time of day blood is drawn and family history of cancer; 2) CEA value obtained were similar between serum or plasma; and 3) Serum CEA is influenced by the history of smoking or cirrhosis of the liver. B. In Cancer: 1) CEA is not useful as a screening test for early GI cancer; 2) CEA may also be elevated in primary cancer of non-GI; 3) CEA level is dependent on stage, but not influenced by the grade of differentiation; 4) Markedly elevated serum CEA (> 25 ng/ml) are highly suggestive of metastatic cancer, particularly with hepatic metastasis; 5) Serum CEA is useful in the detection of metastatic cancer, even in the absence of any other biochemical abnormalities; and 6) The usefulness of CEA in monitoring effects of surgery, radiotherapy and chemotherapy are currently under investigation. C. Summary of the Studies of Secretion of CEA-Like Activity in Man: 1) CEA-like activity is detectable from the secretions of the human gastrointestinal tract; 2) The secretory rate of the CEA-like material was highest in the colonic followed by the pancreatic-biliary secretion. The clinical value of simultaneous measurements of secretion of CEA-like activities in biological fluids and serum CEA remain to be determined.

Significance for Cancer Research (NCP Objective 5 Approach 3)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: January 14, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $140,000

CONTRACT RESEARCH SUMMARY

Title: Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis

Principal Investigator: Name/Address Performing Organization:	Vay Liang W. Go, M.D. Mayo Foundation Rochester, Minnesota 55901

Contract Number: N01-CB-43895
Starting Date: June 15, 1974 Expiration Date: June 14, 1977

Goal: a) To determine comparative values of newer diagnostic tests in the detection of pancreatic cancer; b) To determine whether surgical intervention of a localized pancreatic cancer signigicantly improves survival rate; and c) To further develop newer procedures for the diagnosis of pancreatic cancer.

Approach: Three cooperating institutions will study by a common protocol, patients whose symptomatology and initial studies are suspicious of cancer of the pancreas and warrant exploratory laparotomy. The following special tests listed will be performed prior to surgery: Blood studies include CEA, alpha-fetoprotein and immunoglobulins, duodenal aspiration and study of pancreatic and biliary secretion include cytology of duodenal aspirates, CEA and other substances of potential diagnostic value for detecting cancer, ultrasonic abdominal study, fiberoptic duodenoscopy with retrograde pancreatocholangiography, pancreatic scan with ^{75}Selenomethionine, selective celiac and/or superior mesenteric angiogram and hypotonic duodenography in jaundiced patients if retrograde pancreatography failed. At the completion of all the studies, a surgical consultation for possible exploratory laparotomy will be obtained. At operation, portal blood will be obtained for additional studies (including CEA and alpha-fetoprotein). If cancer of the pancreas is found at surgery, the appropriate surgery will be performed and these patients will be evaluated at 3-month intervals for 3 years. If no diagnosis is made at surgery, the patient will be put into a follow-up group to be evaluated at 6 month intervals for 3 years.

Progress: Since the approval and funding of this study at the Mayo Clinic, we have organized our local teams of experts in various fields and set the protocol in motion. To date, 40 patients have been considered for the protocol and 28 patients have been entered into the study. Clinical data, results of various procedures, tests and the surgical findings have been collected and entered into the common history formed. The data will be analyzed by the Department of Biostatistics.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: January 15, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $151,355

CONTRACT RESEARCH SUMMARY

Title: Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis

Principal Investigator: Name/Address Performing Organization:	Patrick J. Fitzgerald, M.D. Memorial Hospital for Cancer and Allied Diseases New York, New York 10021

Contract Number: NO1-CB-43975
Starting Date: June 1, 1974 Expiration Date: May 31, 1977

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the 5-year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor-associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: In 6 months about 300 patients with gastrointestinal complaints have been studied. Forty-five of these patients (15%) were suspected of having pancreatic cancer and 27 (9%) were operated upon and found to have it. The operative mortality was 4%. Eleven cases (4%) had "curative" resection; 8 of these are living 2-6 months postoperatively. Selective arteriography was the most helpful diagnostic test. Carcinoma *in situ* was found near the primary cancer in 20% of cases. Serum CEA values were related to the extent of the disease. A new antigen (TPA, tumor polypeptide antigen) gave findings similar to those of the serum CEA. About 8000 asymptomatic patients were screened at the Strang Clinic Institute of Preventive Medicine and although 15 cases of gastrointestinal cancer were proven, no case of cancer of the pancreas was found. About 1 case was expected from incidence rates.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: January 29, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $153,293

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer - Early Detection, Localization and Therapy

Principal Investigator: Name/Address Performing Organization:	Myron R. Melamed, M.D. Memorial Hospital for Cancer and Allied Diseases New York, New York 10021

Contract Number: N01-CN-45007
Starting Date: September 1, 1973 Expiration Date: August 31, 1975

Goal: To evaluate sputum cytopathology as a supplement to annual chest x-rays in detecting pulmonary neoplasms at an "early stage" and to evaluate the efficacy of techniques for prompt localization of radiologically occult lung cancer (e.g., before progression to x-ray positive); in general the efficacy of such screening to reduce lung cancer mortality.

Approach: Over a three-year period, 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 years, all to be followed for at least an additional five year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foudation.

Progress: The first subjects were examined early in June, 1974, and during the succeeding months, small numbers of subjects were seen while the computer record keeping system was being modified. Subject recruitment during November, December and January of 1975 had increased substantially, and we expect that it will reach our goal of two hundred per week very shortly. By the end of December, 1974, there were a total of 1,506 subjects screened. Five patients had been referred for thoracotomy, and three have been proven to have cancer. An incidental study on the evaluation of aerosol-induced and spontaneous sputum specimens indicates that there is no difference in the quality of material obtained by hot or cold nebulizers. Spontaneous sputum gave fewer unsatisfactory specimens.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis Site Visit Date: January 30, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $576,665

CONTRACT RESEARCH SUMMARY

Title: Determination of Optimal Frequency of Screening Strategies

Principal Investigator: Name/Address Performing Organization:	Michael J. Brennan, M.D. Michigan Cancer Foundation Detroit, Michigan 48201

Contract Number: N01-CB-53907

Starting Date: Expiration Date:

Goal: To develop a means whereby the optimum frequency of screening can be determined for early cancer for various anatomic sites in order to assure success in such screening as the least possible cost and hazard to the patient and still be effective in reducing death from cancer.

Approach: A team of clinicians, pathologists, statisticians, operations researchers and epidemiologists will carry out extensive investigation and documentation of the level of present knowledge, both empirical and theoretical on the following five factors: 1) the natural history or development of the disease, 2) the incidence of the disease in the target population, 3) the effectiveness (sensitivity and specificity) of the various available screening tests at each stage of the disease, 4) the effectiveness (on mortality) of intervention (treatment during each stage of the disease) and 5) the cost and hazards of the various screening tests. Disease models will be developed and validated for which screening data exists, or must be developed, such as colon, lung, bladder and prostatic cancer. Through parametric analysis these models will be used to suggest general ranges or minimum limits on the performance of screening tests if they are to be effective in early cancer detection and reducing cancer mortality.

Progress: New Contract

Significance for Cancer Research (NCP Objective_____Approach_____)

Project Officer:

Program: Diagnosis Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: Design of an Experiment to Assess the Impact of Multisite Screening on Total Cancer Mortality

Principal Investigator: Name/Address Performing Organization:	Victor A. Gilbertsen, M.D. University of Minnesota Minneapolis, Minnesota 55455

Contract Number: NO1-CB-53906

Starting Date: Expiration Date:

Goal: The goal of this project is to achieve a design that can be put to clinical use in order to effectively reduce cancer mortality.

Approach: A team will be established with expertise in the fields of clinical oncology, radiology, epidemiology and statistics. The experimental design will be developed on the basis of available knowledge of: 1) high-risk factors, 2) quality of screening procedures, 3) variability in the effectiveness of present day treatment, 4) cost, 5) compliance with follow-up examinations and follow through procedures, 6) side effects of treatments, 7) incidence rates, 8) prevalence rates and 9) case fatality rates. The rationale for the experimental design will be expressed in a format which maked explicit the use of available information and accepted assumptions.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer:

Program: Diagnosis Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: The Use of Screening Technique for Blood in the Stool as a Means of Detecting Early Cancer of the Bowel

Principal Investigator: Victor A. Gilbertsen, M.D.
Name/Address University of Minnesota
Performing Minneapolis, Minnesota 55455
Organization:

Contract Number: NO1-CB-53862
Starting Date: Expiration Date:

Goal: The goal of this project is to increase the five-year survival of the patients with large bowel cancer.

Approach: After high risk characteristics are established with the aid of biostatisticians and epidemiologists, patients will be entered into the study in sufficient numbers to give valid comparisons. A control cohort will also be established. Clinical facilities will also be established for diagnosis, treatment and follow-up of large bowel cancer patients. Suitable forms will be designed for conversion to computer storage and subsequent retrieval for evaluation of predisposing factors. The development of a more sophisticated test for human blood will also lead to the design of a common protocol with the collaborating contractor.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or More (over 7-10 mls per 24 hours) of Human Blood in Human Feces

Principal Investigator: Richard E. Rosenfield, M.D.
Name/Address Mount Sinai School of Medicine
Performing New York, New York 10029
Organization:

Contract Number: N01-CB-43870
Starting Date: June 1, 1974 Expiration Date: May 31, 1976

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 mls in 24 hours) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor shall develop a procedure for the detection of human blood in feces by utilizing red blood cell surface antigens specific for human blood. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry and fish diets and be truly negative by the proposed test.

Progress: Artificial mixtures of feces and blood were tested initially to ascertain if added blood could be recovered either as intact erythrocytes or as stromal fragments after hypotonic lysis. The bulk of erythrocytic membranes from intact red cells introduced into a fecal sample can be recovered. In contrast, intact red cells in such a mixture have eluded recovery. Tests for residual antigenicity of stroma recovered from stool have disclosed serious problems from presence in stool of enormous quantities of bacteria, proteases, and apparently neuraminidases. To circumvent these problems, a new solid-phase hemagglutination test is being evaluated. The method, which was not described in our contract, is effective and, in preliminary experiments of artificial mixtures of blood in stool, has permitted us to specifically detect blood in stool. It appears, therefore, that this project may be successful, although the final degree of success will depend upon the sensitivity which can be achieved.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: December 16, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $72,180

CONTRACT RESEARCH SUMMARY

Title: Study of Intrabronchial Deposition of Inhaled Radioopaque Particles

Principal Investigator: Name/Address Performing Organization:	Morton Lippmann, Ph.D. Institute of Environmental Medicine New York University Medical Center New York, New York 10016

Contract Number: N01-CB-33855
Starting Date: November 1, 1972 Expiration Date: March 31, 1975

Goal: To determine the amount of tantalum dust that must be deposited for satisfactory bronchography.

Approach: Using casts of the bronchial tree, tantalum is deposited in various amounts and ways, and the adequacy of deposition assessed by X-rays and other methods. Rates of deposition and distribution from dust clouds with varying particle size and concentration are evaluated in relation to airway dynamics, subject position, and so forth.

Progress: It has been shown in models that optimal bronchography requires about 8 mg/cm^2 of tantalum. This deposition can be produced with dust averaging particles 3 microns in diameter, using a dust generator developed at the University of Pittsburgh, a flow rate of 8 L/min and exposure of 30 min prone and 30 min supine. (Generator modification may permit shorter exposure). The next step will be the determination in humans of the amount of alveolar deposition (an undesirable side effect) and rate of its clearance, not with tantalum, but with a tagged ferric oxide aerosol of equivalent size.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Successful development of tantalum bronchography will greatly improve the diagnostic localization of lung cancer.

Project Officer: I. C. Plough, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $10,092

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues

Principal Investigator: Name/Address Performing Organization:	Raymond Damadian, M.D. Downstate Medical Center, SUNY Brooklyn, New Yrok 11203

Contract Number: N01-CB-43979
Starting Date: June 15, 1974 Expiration Date: June 14, 1976

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.

Approach: To conduct blind studies on approximately 2000 surgical specimens by means of NMR determinations. To determine the extent to which malignant tissue can be detected by NMR in the presence of normal tissue. To explore the feasibility of detecting internal tumors in small laboratory animals.

Progress: NMR studies are being made on four biologically significant atomic nuclei, hydrogen (proton, ^{1}H), potassium (^{39}K), phosphorus (^{31}P) and ^{23}Na (sodium). To date, T_1, T_2 and $T_{1\rho}$ measurements have been made on approximately 600 biological samples. T_1 values of malignant tissues were generally elevated above normal. The difference also appears to hold for the T_2 and $T_{1\rho}$ parameters. NMR studies with ^{39}K, ^{31}P, and ^{23}Na have been limited thus far to tissues from rats and mice. A study of the ^{39}K nucleus is now complete. Tumors are distinguished from normal by the presence of an oscillation in the T_1 plots of tumor tissue. ^{31}P appears to be particularly encouraging. T_1 relaxation times for malignant tumors were 2-3 times the normal values. The degree of overlap in NMR measurements between normal and malignant human surgical tissue is being defined and will affect the value of such measurements for detection and diagnosis.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: February 5, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes that May Presage the Presence of Cancer

Principal Investigator: Name/Address Performing Organization:	Eric A. Barnard, Ph.D. SUNY at Buffalo Buffalo, New York 14214	Tsann Ming Chu, Ph.D. Roswell Park Mem. Inst. Buffalo, N.Y. 14263

Contract Number: NO1-CB-43977
Starting Date: June 1, 1974 Expiration Date: May 31, 1977

Goal: To develop and establish a highly sensitive test for a specifically prostatic form of serum acid phosphatase as a marker for early prostatic cancer.

Approach: It is intended to prepare in bulk the isoenzymes of human prostatic acid phosphatase, by methods previously proven in this laboratory in small-scale work, Both the water-extracted types, which are believed to be secreted in the prostatic fluid, and the detergent-extracted type (obtained by a Tween method in previous work) will be separately examined. Each will be used as an antigen to obtain antibody and other reagents for a radio-immunoassay method, for its specific detection at very low levels in sera. Each will also be studied for any specific binding or inhibitory property that can characterized it at low levels in the presence of other phosphatases.

Progress: Acid phosphatase isoenzymes from normal, hypertrophic prostate glands and carcinoma of the prostate have been isolated and characterized. Isolation of acid phosphatase from serum of patients with prostatic cancer has also been attempted. Levels of acid phosphatase activity in human prostatic fluids have been studied. Antisera against total prostatic acid phosphatase and purified acid phosphatase isoenzyme have been prepared in rabbits and characterized by immunological techniques. A preparation of purified prostatic acid phosphatase isoenzyme has been iodinated with ^{125}I and a radio-immunoassay is being studied. Over 1,000 serum samples from 140 patients with prostatic cancer at different stages of disease have been collected and stored at -75°C for later assay experiments.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: March 17, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $72,583

CONTRACT RESEARCH SUMMARY

Title: Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator: Name/Address Performing Organization:	Harry W. Fischer, M.D. University of Rochester School of Medicine and Dentistry Rochester, New York 14642

Contract Number: N01-CB-43896

Starting Date: June 1, 1974 Expiration Date: May 31, 1976

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time (within a 24 hour period) and without excessive dietary restrictions and without excessive patient discomfort or inconvenience.

Approach: To put to trial combinations of some older methods, or combinations of certain features of older methods, along with the testing of a previously little used method of assisting conversion of solid stool to semi-solid or liquid phase. It is proposed that fecal material be converted to a more liquid form (Embring) and the patient then be assisted to evacuate. The entrance of additional material into the colon from above would be minimized by a certain degree of dietary restriction prior to the definitive portions of the technique aimed at stool conversion and evacuation.

Progress: Evaluation of toxicity of ammonium alum for possible use as a cleansing or barium enema has progressed through (1) gross and histologic study of rat tissue, and (2) enzyme studies in rats after standardized enema-retention techniques and is now being concluded. In vitro testing of various surface active agents on ability to reduce solid fecal material to a more liquid state is beginning. Another method of cleansing the bowel for barium enema, now being tested in a small number of human volunteers, consists of flushing the intestinal tract with a hypotonic balanced salt solution until clear liquid bowel movements indicate the colon is completely cleansed and ready for barium enema examination.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg

Program: Diagnosis Site Visit Date: March 18, 1975

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 74 Funds: $50,500

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes that May Presage the Presence of Cancer

Principal Investigator: Name/Address Performing Organization:	Joshua Lederberg, Ph.D. Stanford University School of Medicine Stanford, California 94305

Contract Number: NO1-CB-43902
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To develop further the uses of gas-liquid chromatography and mass spectrometry instrumentation, under computer management, for the study of derangements of human biochemical metabolism due to neoplastic change; this is to be done for the purpose of identifying new metabolites, or altered levels of known metabolites, which might be diagnostic of cancer.

Approach: (a) To screen urine by GC/MS from control subjects and from individuals suffering from the following forms of cancer:
(1) prostatic cancer
(2) bladder cancer,
(3) Hodgkin's disease, and
(4) various lymphomas.
(b) The quantitation of urinary beta-aminoisobutyric acid levels from cancer patients.
(c) The quantitation of urinary protein amino acid levels in cancer patients.

Progress: Methods are being developed for isolation and quantitation of urinary polyamines which will be investigated for their diagnostic value. For quantitation mass fragmentography with its high sensitivity and specificity is being tested. Deuterated polyamines have been synthesized for use as internal standards in the mass fragmentographic analysis. Isolation methods studies thus far include ion exchange and butanol extraction. Gas chromatography coupled with mass spectrometry has been used to study polyamine derivatives. GC-MS profiles have been determined on 24 cancer patients including 8 with bladder cancer, 6 with non-Hodgkin lymphomas, 3 with prostate cancer, and 7 with leukemias. From these preliminary studies beta-aminoisobutyric acid may prove to have diagnostic significance. Other unidentified urinary components with possible correlation to cancer also have been found. Data from mass fragmentography require computer analysis which is being developed.
Significance for Cancer Research (NCP Objective 5 Approach 4)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: March 3, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $88,108

CONTRACT RESEARCH SUMMARY

Title: Design of an Experiment to Assess the Impact of Multisite Screening on Total Cancer Mortality

Principal Investigator: Dean F. Davies, M. D.
Name/Address University of Tennessee
Performing Memphis, Tennessee 38163
Organization:

Contract Number: N01-CB-53864
Starting Date: Expiration Date:

Goal: The goal of this project is to achieve a design that can be put to clinical use in order to effectively reduce cancer mortality.

Approach: A team will be established with expertise in the fields of clinical oncology, radiology, epidemiology and statistics. The experimental design will be developed on the basis of available knowledge of: 1) high risk factors, 2) quality of screening procedures, 3) variability in the effectiveness of present day treatment, 4) cost, 5) compliance with follow-up examinations and follow through procedures, 6) side effects of treatments, 7) incidence rates, 8) prevalence rates and 9) case fatality rates. The rationale for the experimental design will be expressed in a format which makes explicit the use of available information and accepted assumptions.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer:
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development and Testing of a System to Improve X-Ray Imaging, Contrast and Sharpness by Elimination of Scatter While Retaining the Resolution of Film

Principal Investigator: Name/Address Performing Organization:	Melvin P. Siedband, M.S. University of Wisconsin Madison, Wisconsin 53706

Contract Number: NO1-CB-53914
Starting Date: Expiration Date:

Goal: To remove scatter and thus improve sharpness and contrast which will in turn improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.

Approach: To construct a system that will function in a clinically useful range of exposure times and delivered dose and that can obtain the stated primary goals by mechanical and/or electronic means and should eliminate the need for grids and air gaps. Methods will be developed to determine the quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast and dose reduction. Feasibility should be established during the first year of this contract.

Progress: New Contract

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer:
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds:

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Name/Address Performing Organization:	Cornelius A. Tobias, Ph.D. and Eugene V. Benton, Ph.D. ERDA - Lawrence Berkeley Laboratory University of California, Berkeley Berkeley, California 94720

Contract Number: Y01-CB-40302
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To determine if a proton beam from a high energy (200-400 Mev) source is capable of improving the diagnosis of cancer and of reducing the dosage of ionization radiology.

Approach: Using accelerators available at Berkeley, particularly the 184" cyclotron and the Bevalac heavy-ion accelerator, development of a technique for diagnostic particle radiography of humans is planned by experimental testing of radiologic properties of protons, helium ions and carbon ions; and of detectors such as photographic film and plastic, nuclear-track-recording films. Human diagnostic studies on cancer will proceed with the particles and detectors that offer optimum resolution and contrast at low diagnostic dose levels. Preliminary studies with accelerated oxygen ions and plastic detectors indicate that this system has superior density resolution for the imaging of soft tissues. It is anticipated that particle radiography may materially aid in detection of small soft-tissue tumors, in delineating the extent of invasive tumors that have different density from normal tissue, and in detecting blood clots inside the body. Diagnosis of soft-tissue abnormalities of the brain and direct studies of the formation of atheromatous plaques in large blood vessels will also be attempted.

Progress: Studies to date indicate that higher contrast is achieved with carbon ion beams than with proton beams. Results obtained with the plastic film detector system are promising. The plastic film provides good resolution, requires low dosage, has low scatter, high speed recording and lends itself to complete computer processing. Development of the ion accelerator specifically suitable for clinical use is underway and will involve active participation of clinical radiologists.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: January 20, 1975
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $125,000

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Name/Address Performing Organization:	T. F. Budinger, M.D., Ph.D. ERDA - Lawrence Berkeley Laboratory University of California, Berkeley Berkeley, California 94720

Contract Number: YO1-CB-50304
Starting Date: Expiration Date:

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects is in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labelling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labelled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project algorithms will be developed for computerized reconstruction of clinical data produced by established and newly developed transaxial nuclide projection machines. In addition, techniques for algorithm evaluation and comparison will be developed and applied on suitable materials. Algorithms, written in a high level programming language such as FORTRAN, together with phantom, animal and human emission and transmission projection data and reconstructions will be recorded on nine track industry compatible magnetic tape and delivered to the National Cancer Institute. The National Cancer Institute shall make independent evaluations and disseminate this information to other investigators.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Name/Address Performing Organization:	Lester S. Skaggs, Ph.D. University of Chicago Chicago, Illinois 60637

Contract Number: N01-CB-43918
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To determine if a proton beam from a high energy (200-400 Mev) source is capable of improving the diagnosis of cancer and reducing the dosage of ionization radiology.

Approach: While it is believed that the theoretical studies made and the experimental results already obtained indicate that proton radiography may have great potential as a medical diagnostic tool, it is hoped in the present study to demonstrate this fact conclusively and to provide convincing proof that development of this new modality should proceed with all possible speed. For this purpose, the initial studies at Harvard University will be expanded with the proton beams at Argonne National Laboratory with different characteristics and more sophisticated detection methods. This work will include physical measurements on test objects to define quantitatively the capabilities and limitations of proton radiography. As regards density resolution for situations approximating those to be encountered in actual cases, measurements on tissue specimens will be carried out and the results also will be compared with density measurements on a density column. Finally, when proper equipment becomes available, patients will be examined and the interpretation compared with the best that can be obtained by present day methods in x-ray diagnosis and nuclear medicine. Primary effort will be made in diagnosis of mammary carcinoma where conditions are expected to favor the most rapid evaluation of the efficacy. Some effort will be expended on brain tumors and consideration will be given to other areas such as liver and pancreas.

Progress: Exploratory studies are in progress to determine factors to be considered in developing a properly shielded proton beam suitable for clinical diagnostic use.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: January 22, 1975
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $100,691

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Name/Address Performing Organization:	Sadek K. Hilal, M.D., Ph.D. Columbia University College of Physicians and Surgeons New York, New York 10032

Contract Number: N01-CB-43910
Starting Date: June 30, 1974 Expiration Date: June 29, 1977

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Fifty-one patients in Group I and 4 patients in Group II (total 55) have been studied. Of the 51 patients in Group I, 39 were verified cancer (29 proven tumors, 10 others), 4 were unverified but strongly suspicious of tumor and 8 were unverified and probably not neoplastic. Among the 39 verified cases of Group I there was agreement between CT and nuclear scanning (NS) in 32. In the remaining 7 cases with disagreement, the CT results were positive and NS results negative in 5 cases and vice versa in 2 cases. Among the 33 tumor cases in Group I (29 proven and 4 strongly suspicious cases) there was agreement between CT and NS in 27 and disagreement in 6 cases. In 5 of the latter CT was positive and NS negative.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: February 18, 1975
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $111,013

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Name/Address Performing Organization:	D. Gordon Potts, M.D. Cornell University Medical College New York, New York 10021

Contract Number: N01-CB-43980
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Ninety-two (92) patients in Group I, 7 patients in Group II, and 3 normal subjects (total 102) have been studied. These patients are referred for study by the Departments of Neurology or Neurosurgery at New York Hospital or Memorial Hospital. The isotope scans, EMI scans, angiograms, pneumograms and skull films are being performed using projections that can be readily compared. The magnification and degree of distortion of every view may be determined. Patients who come to autopsy will have giant histological sections prepared to show the entire brain in the planes of the EMI sections. This will permit an accurate comparison of the EMI views with the other studies.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: February 19, 1975
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $119,637

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator:	David O. Davis, M.D.
Name/Address	George Washington University
Performing Organization:	Washington, D.C. 20006

Contract Number: N01-CB-43981

Starting Date: June 30, 1974 Expiration Date: June 29, 1977

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Project became active in early October, 1974. Since that time, a total of approximately 70 patients have been entered into Group I and II. Almost all of those studied in Group I have had contrast studies in addition to plain EMI scan. Radionuclide scans are being evaluated independently of the EMI scan.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.

Program: Diagnosis Site Visit Date: February 14, 1975

Technical Review Group: Diagnostic Radiology Committee

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 74 Funds: $110,335

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Name/Address Performing Organization:	Paul F. J. New, M.D. Massachusetts General Hospital Boston, Massachusetts 02114

Contract Number: N01-CB-43983
Starting Date: June 30, 1974 Expiration Date: June 29, 1975

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: During the current contract year it is estimated that 97 patients will have been entered in Group I and 119 in Group II.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: February 13, 1975
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $119,935

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Name/Address Performing Organization:	John A. Correia, Ph.D. Massachusetts General Hospital Boston, Massachusetts 02114

Contract Number: N01-CB-53859

Starting Date: Expiration Date:

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects appears particularly promising for use with structure-specific labelling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labelled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location and intensity of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project, algorithms will be developed for computerized reconstruction of clinical data produced by novel transaxial nuclide projection machines. Novel radionuclide imaging systems and appropriate algorithms will be emphasized. These algorithms, written in a high level programming language such as FORTRAN, together with appropriate phantom, animal and human projection data and reconstructions, will be recorded on nine track industry compatible magnetic tape and delivered to the National Cancer Institute for independent review and evaluation and dissemination.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.

Program: Diagnosis Site Visit Date:

Technical Review Group: Diagnostic Radiology Committee

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 75 Funds: $130,000

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Name/Address Performing Organization:	Hillier L. Baker, M.D. Mayo Foundation Rochester, Minnesota 55901

Contract Number: N01-CB-43982
Starting Date: June 15, 1974 Expiration Date: June 14, 1977

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: A total of 62 patients have been entered into the protocol on use of CT for diagnosis of brain tumors. Most patients have been in Group I and are about equally divided among categories A, B and C. About 50% of patients in categories A and B have proved to harbor cerebral neoplasms, either primary or secondary, and the majority of these have been proved by surgical exploration or special radiologic examinations. In category C, those symptomatic patients with a low degree of probability of harboring a tumor, we have found only one or two cerebral neoplasms. Three Group II patients per week will be examined for the remainder of the contract period.

Analysis of the differential diagnostic abilities of nuclear and CT scans reveal that the CT scan and its preliminary time appears to be more specific in its diagnostic ability and there is minimal favorable trend in favor of the CT scan as far as detectability of lesions is concerned.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: January 16, 1975
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $119,570

CONTRACT RESEARCH SUMMARY

Title: Construction of a Projection Data Base for Testing Algorithms for Computerized Transaxial Reconstruction

Principal Investigator: Name/Address Performing Organization:	Richard A. Robb, Ph.D. Mayo Foundation Rochester, Minnesota 55901

Contract Number: N01-CB-53857
Starting Date: Expiration Date:

Goal: To obtain information which can be used by radiologists to expedite establishment of new computerized evaluation procedures for improved x-ray detection of early cancer lesions.

Approach: A transaxial x-ray projection data base suitable for testing computerized reconstruction algorithms will be assembled. The data base will include five distinct classes of transaxial planar projection information: simulated data and data from phantoms, excised organs and body specimens, live animals and human subjects. Real projection data will be obtained using x-ray sources and detectors of established physical characteristics and various geometries under controlled experimental conditions. Simulated projection data will be mathematically and stochastically defined in accordance with explicit anatomical, biological and radiological models of normal and pathological physiologic states and of the data acquisition process. Projection data will be digitized and recorded on industry compatible magnetic tape and delivered to the National Cancer Institute, along with appropriate documentation. The National Cancer Institute will furnish the data to other contractors for evaluation, improvement and comparison of reconstruction algorithms.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: November 19, 1974
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Name/Address Performing Organization:	David E. Kuhl, M.D. University of Pennsylvania Philadelphia, Pennsylvania 19174

Contract Number: NO1-CB-53913
Starting Date: Expiration Date:

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects is in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labelling with a wide array of radioactive compounds. Emission imaging permits not only static reconstruction but also quantitative estimates of time-course movements of labelled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location of radioactivity in small body volumes that would be lost or obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project, algorithms will be developed for emission circumferential aperture reconstruction tomography (ECART) from clinical projection data collected by a new transaxial coded ring aperture radionuclide scanner with high count efficiency.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $72,000

CONTRACT RESEARCH SUMMARY

Title: Development and Evaluation of Computerized Transaxial X-Ray Reconstruction

Principal Investigator: Name/Address Performing Organization:	Sadek K. Hilal, M.D., Ph.D. Presbyterian Hospital Columbia-Presbyterian Medical Center New York, New York 10032

Contract Number: N01-CB-53858
Starting Date: Expiration Date:

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification and localization of cancer pathology in the chest, abdomen and other anatomical regions, with potential low risk to the patient.

Approach: In the initial year of the study, equipment and displays will be developed and fabricated for computerized transaxial x-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two year endeavor, clinical trials of the equipment will be pursued on suitable human subjects and results compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithms modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: November 20, 1974
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $300,000

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial X-Ray Reconstruction

Principal Investigator: Name/Address Performing Organization:	Gabor T. Herman, Ph.D. Research Foundation of SUNY Albany, New York 12224

Contract Number: NO1-CB-53860
Starting Date: Expiration Date:

Goal: To improve detection and diagnosis of early cancer lesions by means of improved radiologic imaging techniques in involving new mathematical algorithms for computerized transaxial x-ray reconstruction.

Approach: Algorithms will be developed for computerized three-dimensional reconstruction of x-ray absorption techniques from projection data collected via various sources and detector geometries. The reconstruction will minimize the anatomical and densitometric distortion and artifactual effects in regions of medical interest and decrease both scan time and radiation risk of the patient. Criteria also will be developed for evaluating efficacy of the algorithms. The algorithms will be written in a high level programming language such as FORTRAN and assembled in a library which takes advantage of input and output commonalities and is modular and expandable. The library of algorithms plus suitable mathematical and actual test data and results will be written on nine track industry compatible tape and delivered to the National Cancer Institute for distribution to other investigators in the field.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: November 19, 1974
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Arthur J. Present, M.D. University of Arizona Tucson, Arizona 85724

Contract Number: N01-CN-55097

Starting Date: October 9, 1974 Expiration Date: October 3, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.

Program: Cancer Control Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Institute Director and Executive Committee

FY 75 Funds: $185,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Myron Moskowitz, M.D. University of Cincinnati College of Medicine Cincinnati, Ohio 45229

Contract Number: NO1-CN-35024
Starting Date: June 29, 1973 Expiration Date: June 28, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	7,826
	Biopsies recommended	1,146
	Biopsies performed	400
	Cancers found	58

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: December 6, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $170,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Richard G. Lester, M.D. Duke University Durham, North Carolina 27710

Contract Number: N01-CN-45064
Starting Date: February 15, 1974 Expiration Date: February 14, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	4,536
	Biopsies recommended	130
	Biopsies performed	87
	Cancers found	10

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $130,354

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Harold J. Isard, M.D. Albert Einstein Med. Ctr. Philadelphia, Pa. 19141	Marc Lapayowker, M.D. Temple University Hosp. Philadelphia, Pa. 19140

Contract Number: N01-CN-45058
Starting Date: January 2, 1974 Expiration Date: January 1, 1976

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

		Einstein	Temple
Progress:	Total women screened	2,770	1,886
	Biopsies recommended	69	68
	Biopsies performed	44	59
	Cancers found	9	9

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: March 5, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 75 Funds: $180,737

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Robert L. Brown, M.D. Robert L. Egan, M.D. Emory University Atlanta, Georgia 30322	A. Hamblin Letton Georgia Baptist Hosp. Atlanta, Georgia 30312

Contract Number: N01-CN-35016
Starting Date: May 9, 1973 Expiration Date: May 8, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

		Emory	Georgia Baptist
Progress:	Total women screened	1,475	5,017
	Biopsies recommended	32	203
	Biopsies performed	27	126
	Cancers found	4	23

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. G.B. December 3, 1974
Program: Cancer Control Site Visit Date: E.U. December 4, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $177,341

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	William L. Donegan, M.D. Ellis Fischel State Cancer Hosp. Columbia, Missouri 65201

Contract Number: N01-CN-45095
Starting Date: June 1, 1974 Expiration Date: May 31, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	2,928
	Biopsies recommended	99
	Biopsies performed	59
	Cancers found	11

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $174,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Margaret Abernathy, M.D. Georgetown University Washington, D.C. 20007

Contract Number: N01-CN-45062
Starting Date: February 7, 1974 Expiration Date: February 6, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and"early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	720
	Biopsies recommended	31
	Biopsies performed	1
	Cancers found	0

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $135,318

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Morton J. Goodman, M.D. Good Samaritan Hosp. and Med. Center Portland, Oregon 97201

Contract Number: N01-CN-45088
Starting Date: June 1, 1974 Expiration Date: May 31, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis canbe detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	3,289
	Biopsies recommended	58
	Biopsies performed	28
	Cancers found	6

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $169,754

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Philip Strax, M.D. Guttman Breast Institute New York, New York 10013

Contract Number: N01-CN-35004
Starting Date: April 18, 1973 Expiration Date: April 17, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography,mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:	Total women screened	21,992
	Biopsies recommended	705
	Biopsies performed	358
	Cancers found	86

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: October 4, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $255,720

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Donald C. Young, M.D. Iowa Lutheran Hospital Des Moines, Iowa 50316

Contract Number: N01-CN-45067
Starting Date: February 27, 1974 Expiration Date: February 26, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	2,073
	Biopsies recommended	33
	Biopsies performed	8
	Cancers found	2

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $122,366

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Loren J. Humphrey, M.D., Ph.D. Kansas Univ. Med. Center Kansas City, Kansas 66103

Contract Number: NO1-CN-35026
Starting Date: June 19, 1973 Expiration Date: June 18, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts, will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	5,878
	Biopsies recommended	172
	Biopsies performed	156
	Cancers found	24

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: January 23, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $165,170

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Condict Moore, M.D. University of Louisville Louisville, Kentucky 40201

Contract Number: N01-CN-35012
Starting Date: April 17, 1973 Expiration Date: April 16, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	6,831
	Biopsies recommended	579
	Biopsies performed	183
	Cancers found	41

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: December 5, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $163,481

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Thomas Carlile, M.D. Virginia Mason Hospital Seattle, Washington 98101

Contract Number: N01-CN-35028
Starting Date: June 15, 1973 Expiration Date: June 14, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:

Total women screened	7,213
Biopsies recommended	120
Biopsies performed	118
Cancers found	52

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: January 14, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $183,536

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Robert Schweitzer, M.D. Samuel Merritt Hospital Oakland, California 94609

Contract Number: N01-CN-45068
Starting Date: February 25, 1974 Expiration Date: February 24, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:

Total women screened	2,243
Biopsies recommended	261
Biopsies performed	55
Cancers found	15

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $142,007

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Barbara Threatt, M.D. University of Michigan Ann Arbor, Michigan 48104

Contract Number: NO1-CN-45049
Starting Date: March 18, 1974 Expiration Date: March 17, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	1,775
	Biopsies recommended	76
	Biopsies performed	?
	Cancers found	2

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $133,333

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Elisabeth Ward, M.D. Mountain States Tumor Institute Boise, Idaho 83702

Contract Number: N01-CN-35029
Starting Date: June 25, 1973   Expiration Date: June 24, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	6,748
	Biopsies recommended	141
	Biopsies performed	123
	Cancers found	32

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control   Site Visit Date: January 15, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $181,463

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Benjamin Rush, M.D. College of Medicine and Dentistry of New Jersey Newark, New Jersey 07103

Contract Number: N01-CN-35006
Starting Date: June 29, 1973 Expiration Date: October 27, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermograpy, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breastcancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	6,882
	Biopsies recommended	100
	Biopsies performed	65
	Cancers found	28

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: January 24, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $183,333

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	JoAnn D. Haberman, M.D. University of Oklahoma Health Science Center Oklahoma City, Oklahoma 73190

Contract Number: N01-CN-35020
Starting Date: June 18, 1973 Expiration Date: June 17, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:

Total women screened	7,552
Biopsies recommended	377
Biopsies performed	?
Cancers found	26

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: January 17, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $183,333

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Fred I. Gilbert, Jr., M.D. Pacific Health Research Inst. Honolulu, Hawaii 96813

Contract Number: NO1-CN-45046
Starting Date: December 19, 1973 Expiration Date: December 18, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 women in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened for five screenings and to be followed annually for five years thereafter.

Approach; Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and evenhopefully to "cure" breast cancer.

Progress:		
	Total women screened	5,397
	Biopsies recommended	107
	Biopsies performed	146
	Cancers found	12

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: June 12, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $171,966

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Bernard Fisher, M.D.
Name/Address University of Pittsburgh
Performing Pittsburgh, Pennsylvania 15261
Organization:

Contract Number: N01-CN-45065
Starting Date: February 15, 1974 Expiration Date: February 14, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:	Total women screened	1,310
	Biopsies recommended	12
	Biopsies performed	5
	Cancers found	2

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $133,762

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Herbert P. Constantine, M.D. Rhode Island Hospital Providence, Rhode Island 02902

Contract Number: N01-CN-45096
Starting Date: May 28, 1974 Expiration Date: May 27, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	775
	Biopsies recommended	18
	Biopsies performed	5
	Cancers found	3

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $180,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Duncan L. Moore, M.D. St. Joseph's Hospital Houston, Texas 77002

Contract Number: N01-CN-55100
Starting Date: October 1, 1974 Expiration Date: September 30, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $185,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Marvin V. McClow, M.D. St. Vincent's Medical Center Jacksonville, Florida 32203

Contract Number: N01-CN- 55210
Starting Date: June 20, 1973 Expiration Date: December 16, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:	Total women screened	5,523
	Biopsies recommended	207
	Biopsies performed	115
	Cancers found	26

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: March 7, 1975
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $180,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Lewis W. Guiss, M.D. University of Southern Calif. Los Angeles, California 90033

Contract Number: N01-CN-45098
Starting Date: June 25, 1974 Expiration Date: June 24, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society(in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	941
	Biopsies recommended	2
	Biopsies performed	0
	Cancers found	0

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $176,194

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Henry Burko, M.D. Vanderbilt Univ. Hospital Nashville, Tennessee 37232

Contract Number: N01-CN-55099
Starting Date: July 24, 1974 Expiration Date: July 23, 1975

Goal: To have the American Cancer Society though its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 75 Funds: $185,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Leslie W. Whitney, M.D. Wilmington Medical Center Wilmington, Delaware 19805

Contract Number: N01-CN-45047
Starting Date: January 1, 1974 Expiration Date: December 31, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	5,117
	Biopsies recommended	102
	Biopsies performed	82
	Cancers found	17

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group:
FY 74 Funds: $130,652

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Name/Address Performing Organization:	James E. Youker, M.D. The Medical College of Wisconsin Milwaukee, Wisconsin 53226

Contract Number: N01-CN-35018
Starting Date: June 18, 1973 Expiration Date: June 17, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to a project of thermography, mammography and physical examination for the detection of breast cancer and be rescreened annually for five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:		
	Total women screened	5,572
	Biopsies recommended	118
	Biopsies performed	114
	Cancers found	28

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date: October 2, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $173,958

CYTOLOGY AUTOMATION CONTRACT PROGRAM

ANNUAL REPORT SUMMARY

July 1, 1974 to June 30, 1975

The Committee on Cytology Automation was established in July, 1972 to advise the Director, Division of Cancer Biology and Diagnosis, National Cancer Institute on all matters pertaining to Cytology Automation. Specifically, the Committee (1) serves as advisors to National Cancer Institute on the state of the art in scientific and technical areas which bear on automation, and (2) advises the National Cancer Institute on plans and implementation in operational terms for National Cancer Institute's contractual support of developing automation in cytopathology.

The Committee determined that six areas are within the mission of the Commettee:

1. Specimen Collection
2. Specimen Preparation
3. Sensing-screening Systems
4. Decision-diagnostic Systems
5. Specimen Sources
6. System Evaluation

At the present time fourteen contracts in cytology automation are in operation. Three of the earliest contracts emphasized instrument development in categories three and four above. These instruments being evaluated include a multiparameter cell analysis and sorting system at Los Alamos Scientific Laboratory of ERDA (zero resolution); a high-resolution image processing system at the University of Chicago and an intermediate-resolution flow analysis sytem at the University of Rochester.

Major expansion of the Cytology Automation program during FY 1974 and continuing into FY 1975 was in areas one and two above with the funding of eight new contracts to investigate the problems associated with preparation and stoichiometric staining of clinical specimens. This area has consistently been the stumbling block on which technically excellent instruments have failed in clinical application. The contracts include both development of new techniques and application of techniques from other areas such as tissue culture to the specific problems presented by clinical cytopathologic specimens.

In order to evaluate the biological requirements for a successful automated system, a contract has begun at the University of Chicago to quantify the numbers and types of cells present on cytologic smears from endocervix, ectocervix and vagina (areas one and two above). The data provided by this study will provide a foundation on which to evaluate compatibility of automated system specimen preparation with preservation of diagnostic value of the specimen.

Finally, the Cytology Automation program has funded contractual support to ensure the investigators working in the program an adequate supply of clinical material with which to test techniques and instruments.

Five other projects concerning Cytology Automation are pending. All have been approved.

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Stains and Other Optical Markers Useful for Gynecologic Cytopathology Specimens

Principal Investigator: Name/Address Performing Organization:	Seymour West, Ph.D. University of Alabama Birmingham, Alabama 35294

Contract Number: N01-CB-43960
Starting Date: June 30, 1974 Expiration Date: June 29, 1975
Goal: Evaluate the applicability of biophysical instrumental analysis to clinical cytopathologic pre-screening.

Approach: The Contractor will apply considerable general experience with biophysical techniques to the evaluation of the possible usefulness of these techniques in clinical cytopathologic pre-screening. These techniques employ acridine orange as a molecular probe and evaluation includes absorption and emission spectra, circular dichroism, optical rotatory dispersion. Preliminary investigation (feasibility study) of lectin-agglutination of clinical specimens will also be performed.

Progress: Emission spectra of acridine - orange stained cervical, vaginal cells have been under investigation. The spectra are recorded rapidly enough that fading of fluorescence is not a factor in the qualitative analysis. Dye-intracellular biopolymer complexes are studied by fluorescence measurements as possible markers for normal and abnormal cells. Intracellular macromolecules bind differently with acridine - orange.

Methods for detecting and quantitating cell agglutination have been under development. Some tumor cells have been shown to undergo agglutination in the presence of many lectins. The clinical gynecologic specimens are tested for cell agglutination and quantitatively and qualitatively analyzed. The selective agglutination of malignant cells would greatly enhance the early diagnosis by automated instrument.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Identification of new machine sensible properties suitable for distinguishing neoplastic from non-neoplastic human cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: January 22, 1974; November 22, 1974 (Dr. Bunnag)
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $299,790

CONTRACT RESEARCH SUMMARY

Title: Studies and Investigations of the Diagnostic Value of Gynecologic Cytopathology Samples

Principal Investigator: Name/Address Performing Organization:	Marluce Bibbo, M.D., Sc.D. University of Chicago Chicago, Illinois 60637

Contract Number: N01-CB-43919

Starting Date: June 15,1974 Expiration Date: June 14, 1975

Goal: To determine the cellular content of cytologic samples from vagina, ectocervix and endocervix of patients with various clinical conditions.

Approach: The principal investigator is using the large volume of Pap smears available at the University of Chicago, which have been collected over the past 18 years, for a retrospective study of the cellular content of the smears. The smears are being examined and counted for relative numbers of epithelial and inflammatory cells of all types from normal through invasive carcinoma cases. It will be noted if cells appear singly or as part of a cluster and if the cells are degenerate or well preserved. The classification will be according to that of the World Health Organization.

Progress: Twenty-eight cases have been counted which include two normal cases, 10 cases of severe dysplasia, 15 cases of carcinoma in situ and one case of poorly differentiated squamous carcinoma. Quality control studies have been performed to insure reproducibility of results.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
It is essential in designing an automated screening system to know the content of the samples to be screened.

Project Officer: Mary Cassidy

Program: Cytology Automation Site Visit Date: February 20, 1974

Technical Review Group: Committee on Cytology Automation

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 74 Funds: $77,030

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Approaches to the Problem of Markers Applicable to Gynecologic Cytopathology Specimens

Principal Investigator: Name/Address Performing Organization:	Professor Torbjorn Caspersson, M.D. Karolinska Institute Stockholm, Sweden

Contract Number: N01-CB-43945
Starting Date: June 30, 1974 Expiration Date: June 29, 1975
Goal: Automation of clinical cytologic screening and diagnosis, concomitant with improvement in diagnostic accuracy and throughput.

Approach: Dr. Caspersson has pioneered the development of innovative methods of investigative cytology and cytogenetics. Under this contract, he will develop and evaluate prototype machines for automatically measuring cytologic features of clinical gynecologic specimens using high resolution scanning microscopy with absorption ultraviolet-spectrophotometry, interferometry and cytofluorometry, individually and in combination.

Progress: A two parameter instrument has been constructed. The instrument consists of computer controlled servomotor driven scanning microscope, microspectrophotometer and microinterferometer. The scanning microscope is used to examine and select cells and has the capability to recall the previously chosen cells for reexamination and analysis. The integrated instrument permits microspectrophotometric measurements in the UV and/or visible range, followed by microinterferometric measurements on the same cell while the cell remains in the same microscope field of vision. Modification and improvement are being made including incorporation of image intensifier - TV system.

Qualitative measurements of the various cell types of the female genital tract will yield multiparameter data which can be used as discriminators for these different cell types by automated cytology instrument.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Development of very rapid high resolution multiple modality automated scanning microscopes will advance image processing approaches for cytologic screening and diagnosis, and promote better understanding of normal, precancerous and cancerous states.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: February 27,197
Technical Review Group: Committee for Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $38,000

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens

Principal Investigator:	Paul Todd, Ph.D.
Name/Address	Pennsylvania State University
Performing	University Park, Pennsylvania 16802
Organization:	

Contract Number: N01-CB-43984
Starting Date: June 30, 1974 Expiration Date: June 29, 1975
Goal: Investigation of new techniques for preparing and marking cells for automated pre-screening of clinical cytology specimens.

Approach: Cell electrophoresis, both micro and bulk, will be investigated. Heavy meromyosin binding to α-filaments in normal cells of the gynecologic tract will be investigated. Potential utility of HSV II antigens in distinguishing normal from abnormal human cells will be explored. A number of enzymatic techniques for cell dispersal will be investigated and analyzed.

Progress: Heavy meromyosin has been prepared and purified from chicken breast muscle. Testing of meromyosin binding is in progress. Animal model systems indicate that malignant cell surface changes are such that there is surface binding. Human clinical specimens are being tested. Antibodies against the heavy fragment are being prepared to be used as possible markers for abnormal cells.

Antisera are collected and used to seek the non-virion antigen, Ag-4. Isolation of HSV non-virion antigen is under way. The antigen - antibody reaction of abnormal cells is another marker being explored.

Cell electrophoresis has been applied to many cell types including virus-transformed cells. Results indicate that cell surface alterations are sufficient to influence the cell migration rates. Cell electrophoresis is being used to differentiate normal and abnormal cells of the female genital tract.

Many enzymes have been applied to dissociate cells. Enzymes are used one at a time or in combination of a few enzymes. Enzyme analysis and purification is also being done to ensure the qualitative analysis. No single enzyme has been found to effectively dissociate cells.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Cell preparative techniques and exploitable markers hold the key to automation of cytology screening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: April 4, 1974
Technical Review Group: Committee on Cytology Automation October 24, 1974
Relevance Review Group: Cancer Diagnosis Steering Committee (Dr. Bunnag)
FY 74 Funds: $233,906

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens in Suspension

Principal Investigator: Name/Address Performing Organization:	Leopold G. Koss, M.D. Montefiore Hospital and Medical Center Bronx, New York 10467

Contract Number: N01-CB-43963

Starting Date: June 1, 1974 Expiration Date: May 31, 1975

Goal: To obtain monodisperse cell suspensions from gynecologic cytopathology specimens for use in flow-through systems being developed for cytology automation.

Approach: The principal investigator will study the natural cell attachments and various ways of disrupting these attachments. Electron microscopy will be used to evaluate the effectiveness of the cell attachment (chiefly desmosome) disruption.

Progress: Vaginal pool aspirates from 27 patients of varying ages have been studied electron microscopically to examine the cell adhesions. Extensive interdigitations and typical desmosomes were seen in the squamous cells. Several desmosome-forming culture lines are being studied with electron microscopy. Cell samples from the female genital tract either suspended in normal saline or fixed in 1% glutaraldehyde were treated with the following dispersing agents: trypsin (0.25%), EDTA (0.1%), collagenase (0.25%) and sodium desoxycholate (1%). The dispersing agents had no effect on the glutaraldehyde-fixed samples and the dispersal of cell clumps was inadequate in the saline suspension samples.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy

Program: Cytology Automation Site Visit Date: February 7, 1974

Technical Review Group: Ad Hoc Committee

Relevance Review Group: Cancer Diagnosis Steering Committee

FY 74 Funds: $183,740

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathologic Specimens in Suspension and on Slides

Principal Investigator: Name/Address Performing Organization:	Walter E. Tolles, Ph.D. SUNY - Downstate Medical Center Brooklyn, New York 11203

Contract Number: N01-CB-43955
Starting Date: June 1, 1974 Expiration Date: May 31, 1975
Goal: To obtain a monodisperse cell sample from gynecologic cytologic specimens for use in flow-through and image processing systems being developed for cytology automation.

Approach: The emphasis will be on lytic and enrichment techniques. Chemical and enzymatic agents will be used to break up clusters as well as mechanical and ultrasonic shearing techniques. Rate-zonal sedimentation will be used as an enrichment technique.

Progress: An automatic syringer has been fabricated and analysis cell disaggregation and cell damage has been accomplished in five cervical specimens. Collagenase treatment of cervical samples has been attempted and best results obtained from unfixed samples suspended in saliva. Rate zonal sedimentations have been made on fifty cervical samples and the distributions of cell types in the various fractions has been quantified.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: February 8, 1974
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $98,611

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigators: Mr. Arthur Hontz and Earl Greenwald, M.D.
Name/Address Performing Organization: Temple University Medical School
and Hospital
Philadelphia, Pennsylvania 19140

Contract Number: N01-CB-43937
Starting Date: June 30, 1974 Expiration Date: June 29, 1975
Goal: Evaluation of multiparameter cell sorter designed for cytology automation.

Approach: The Contractor shall deliver to the Laboratory of Pathology 752 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The Contractor is delivering approximately 82 samples per month to the NCI. The range of sample types has been from normal through invasive carcinoma. These samples are being used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: July 11, 1974
Technical Review Group: Committee on Cytology Automation (Ms Cassidy,
Relevance Review Group: Cancer Diagnosis Steering Committee Dr. Bunnag)
FY 74 Funds: $32,300

CONTRACT RESEARCH SUMMARY

Title: Immunological, Enzymatic and Other Biochemical Markers for Detection of Abnormal Cervical Cells on Slides or in Suspensions by Optical Methods

Principal Investigator: B. D. Halpern, Ph.D.
Name/Address Polysciences, Inc.
Performing Warrington, Pennsylvania 18976
Organization:

Contract Number: N01-CB-43943
Starting Date: June 1, 1974 Expiration Date: May 31, 1975
Goal: Chemical synthesis and preliminary testing of markers for clinical specimens.

Approach: The Contractor will apply his extensive experience in biochemical synthesis to produce absorptive and flourescent dyes to stoichiometrically quantitate macromolecules, enzymatic activity and antigen-antibody systems suggested by basic scientific research to be of potential utility in distinguishing human malignant and pre-malignant cells from non-neoplastic cells in automated cytopathologic screening instruments.

Progress: Fluorescent labeled microspheres have been synthesized. Specific antibodies will be conjugated to these spheres for a possible use as markers for abnormal cells in the direct or indirect immunofluorescent test.

Specially coated glass slides have been made. The slides are capable of binding γ- globulin and will be used for abnormal cell enrichment (on slides) by interaction of these binding sites and antibody - conjugated abnormal cells.

Antibodies specific for cervical carcinoma cell surface antigens are being produced for use in the above procedures.

Synthesis of several new fluorescent macromolecular stains has begun, with specificity oriented toward RNA species and certain related molecules, such as RNA polymerase. These stains are to be used with other well-defined stains to acquire multi-parameter quantitative and qualitative measurements to differentiate normal and abnormal cells by instruments now operational at other contractor's laboratories.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Chemical synthesis and verification of stoichiometric stains useful for clinical use in screening cytopathology specimens.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: January 25, 1974;
Technical Review Group: Committee on Cytology Automation October 25, 1974
Relevance Review Group: Cancer Diagnosis Steering Committee (Dr. Bunnag)
FY 74 Funds: $158,270

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Information Requirements for Image Processing of
Cell Samples as Basis for Development of Automated Cell Recognition
System

Principal Investigator: Name/Address Performing Organization:	George L. Wied, M.D. University of Chicago Chicago, Illinois 60637

Contract Number: N01-CB-33873
Starting Date: May 31, 1973 Expiration Date: April 30, 1976
Goal: Automation of clinical cytologic screening and diagnosis, focusing on definition of information requirements and criteria for high throughput, high resolution image processing-based decision making.

Approach: The Contractor shall evaluate information requirements for characterization and recognition of normal, premalignant and malignant cells from the human female genital tract by means of digital image processing computer feature extraction applied to high resolution, multicolor digital images of clinical specimens. The cytologic material will include 15 main categories with at least 100 cells each, from different subjects, so as to include the effects of intra- and inter-individual variation, physiological age, pregnancy, viral infection, and specific and non-specific inflammatory reactions. Robustness of cytologic features and discrimination logic will be assessed as a function of minor variations in staining and fixation procedures.

Progress: Digitized cell images have been compiled. These data are stored on tapes and will be made available to other contractors for further analyses. These cells are from the various cell types encountered in the exfoliated cell specimens from the female genital tract. Image editing system is operational. Stored cleaned cell images will also be made available for other contractors to analyze with their own algorithms.

Further details on scanned cells are being done using 3-color scanner. The 3-color data are admitted from the Zeiss Axiomat to the PDP-8i, displayed on the VT05 storage scope for quality control, stored on DEC tape and eventually carried to the PDP-10. More specimens are being scanned and data stored. The 3-color scanner should enrich the data base for cell image processing.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
A rapid, high-resolution, multi-spectral digital scanner for clinical gynecologic screening and diagnosis will promote better health care delivery and therapy monitoring for the populations at large and at high risk.
Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: November 20, 1972
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $120,405

CONTRACT RESEARCH SUMMARY

Title: Development and Evaluation of New Stains and Other Optical Markers Useful for Cytopathologic Specimens in Suspension

Principal Investigator: Name/Address Performing Organization:	Brian H. Mayall, M.D. Lawrence Livermore Laboratory Livermore, California 94550

Contract Number: Y01-CB-40300
Starting Date: June 1, 1974 Expiration Date: May 31, 1975
Goal: Development and preliminary clinical testing of stains useful in differentiating normal from non-normal cytopathological material.

Approach: Utilizing their unique combination of flow-sorting and image-processing expertise, the investigators will evaluate specimen preparation and staining techniques on clinical samples. The ability to evaluate specimens at both zero and medium to high resolution will assure the mutual compatibility of preparative techniques and safeguard the diagnostic value of samples prepared for automated systems.

Progress: Clinical specimens have been collected and used for production of monodisperse cell suspensions. The cells are treated by mechanical means, e.g., syringing and chemicals. N-toxin has been found to be more effective than other enzymes. More testing is needed using both mechanical and enzymatic treatments.

Cells of various types have been photographed and mapped for quantitative cytochemistry. The approach ensures that specific cell types are analyzed as the CYDAC is employed to digitize and compile data on these cells. Correlation between data and selected cells can be made since each cell was previously photographed and recorded. Cells have been stained with toluidine blue, naphthol yellow S and alkaline fast green.

Cells in suspension are also quantitatively analyzed by the LLL flow microfluorometer. Data have been obtained pertaining to different fluorescent properties of the various cell types. More samples are being analyzed. Light scattering properties of cells are also being explored.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Development and evaluation of techniques to rapidly screen cell populations for pre-malignant and malignant cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: February 1, 1974; October 31, 1974 (Dr. Bunnag)
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $331,670

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Stains and Other Optical Markers Useful for Clinical Cytopathological Specimens in Suspension

Principal Investigator:	Robert C. Leif, Ph.D.
Name/Address	Papanicolaou Cancer Research Institute
Performing	Miami, Florida 33123
Organization:	

Contract Number: N01-CB-43962
Starting Date: June 30, 1974 Expiration Date: June 29, 1975
Goal: Investigation of new markers and techniques of specimen preparation for clinical cytopathologic screening.

Approach: The investigator will extend the technique of centrifugal cytology to produce improved specimens for analytic instrumentation. Techniques for dialysis staining, heavy metal chelating fluorochromes and immunofluorescence will be investigated as preliminary feasibility studies.

Progress: Studies are in progress using centrifugal cytology to monitor dissociation of cells previously treated by various chemicals. The chemical of choice so far has been dithiothreitol. However, much testing is to be done and combination of other chemicals may be necessary.

An improved pair of centrifugal cytology buckets has been constructed; they should significantly aid in monitoring cell dissociation.

Antibodies have been prepared against HSV-2 and also against cervical carcinoma antigen. The antibodies will be used as possible markers for abnormal cells.

A europium chelate with a narrow band emission spectrum and a primary amino group has been synthesized. Several β-diketonato complexes have been characterized as to solubility and emission spectra. These fluorochromes will be explored for cytology automated instrument in detecting various cell types.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers and preparative techniques for clinical specimens will improve the prospects for more rapid automation of clinical cytopathologic pre-screening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: March 11, 1974;
Technical Review Group: Committee on Cytology Automation December 20, 1974
Relevance Review Group: Cancer Diagnosis Steering Committee (Dr. Bunnag)
FY 74 Funds: $249,774

CONTRACT RESEARCH SUMMARY

Title: Development of Hematoxylin Substitutes for Diagnostic Staining of Tissues and Exfoliated Cells.

Principal Investigator: Ralph D. Lillie, Ph.D.
Name/Address Louisiana State University
Performing Baton Rouge, Louisiana 70586
Organization:

Contract Number: N01-CB-43912
Starting Date: January 11, 1974 Expiration Date: January 10, 1975
Goal: Development of hematoxylin substitutes for diagnostic staining of tissues and exfoliative cells.

Approach: 1. Synthesize various classes of mordant dyes or obtain same from either specific manufacturers or from the archives of the Biological Stain Commission.

2. Perform studies on various tissues with combinations of dyes obtained to achieve staining in accordance with criteria satisfactory to the Project Officer and to the Biological Stain Commission's certification criteria.

3. Procure samples of possibly suitable mordant dyes offering blue or black mordant colors which may be suitable to replace hematoxylin in one or more of its histological staining uses.

4. Subject such samples to the usual tests applied by the Biological Stain Commission for the certification of hematoxylin.

5. Apply favorable test results to routine surgical diagnostic material in Papanicolaou smear diagnosis and in other staining procedures applicable to the differential diagnosis of various cancers, evaluating results in each case in comparison of previously standard hematoxylin procedures and with the best of the previously proposed substitutes.

Progress: Synthesis and testing are on schedule as outlined above.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Stain development is essential to the cytological diagnosis of cancer.

Project Officer: M. F. Stanton, M.D.
Program: Cytology Automation Site Visit Date: Deferred
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $36,690

CONTRACT RESEARCH SUMMARY

Title: Application of High-Speed Flow System Methods of Cell Analysis and Sorting to Cancer-Related Cell Populations

Principal Investigator: Name/Address Performing Organization:	J. A. Steinkamp, Ph.D. AEC-Los Alamos Scientific Laboratory Los Alamos, New Mexico 87115

Contract Number: Y01-CB-10055
Starting Date: June 1, 1974 Expiration Date: May 31, 1975
Goal: Automation of clinical cytologic screening and diagnosis.

Approach: A multiparameter sorter has been developed and is being tested by the contractor to measure and analyze cytobiological parameters related to malignancy. With both model tumor lines and human squamous specimens, this instrument has separated successfully samples which have as few as 8% tumor cells on the basis of DNA content alone. The goals of the present project are biomedical rather than bioengineering. The present effort centers specifically on the development of parameters or combinations of parameters which characterize malignancy, development of new sample preparation and staining techniques suitable for analysis by the sorters, the investigation of lectin binding to surfaces of normal and abnormal squamous cells, the investigation of light scattering and time-of-flight properties of squamous cells.

Progress: The major biological thrusts of the program for the past year include application of the acriflavin - Feulgen procedure as a DNA stain for cervical-vaginal cells, the use of 9-hydrazine acridine as a specific marker for aldehyde groups on DNA, evaluation of acridine orange as a rapid stain, development of mithramycin as a rapid DNA stain, continued evaluation of the collagenase dispersal procedure, and continued development of the DNA-protein staining technique. Instrumentation development has included improved light-scatter signal resolution and a LED strobe lamp-driver unit for the cell sorters; construction of a prototype flat flow chamber, a time-to-amplitude converter, a dual-channel logarithmic amplifier, and the development of a 32-element circular ring photodiode array with associated fast log amplifiers and computer interface, computer-controlled analysis and cell sorting capability, and electronics for cell doublet discrimination.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officer: B. J. Fowlkes
Program: Cytology Automation Site Visit Date: January 18, 1973
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $333,300

CONTRACT RESEARCH SUMMARY

Title: Investigation of a Slit-Scan Technique as a Basis for an Automated Prescreening System for Cancer Detection in Cytology

Principal Investigator: Leon L. Wheeless, Jr., Ph.D.
Name/Address University of Rochester
Performing Rochester, New York 14642
Organization:

Contract Number: N01-CB-33862
Starting Date: March 19, 1974 Expiration Date: March 18, 1975
Goal: Automation of clinical cytologic screening and diagnosis

Approach: The Principal Investigator will continue his effort in the areas of sample preparation and cell behavior in flow-through systems. The data base on slit-scan contours of various normal and abnormal cells will continue to be expanded as well as the information on artefact and bi-nucleated cell recognition. Additional improvements will be made on the slit-scan flow system to improve resolution.

Progress: The study of sample preparation techniques has shown some promising leads especially in the area of mucus dissolution, artefact recognition and cell dispersion by mechanical means. The slit-scan flow-system is being tested and another one is being built to test alterations for potential use on the first instrument. A large data base on bi-nucleated cell recognition has been established and the potential hazard of bi-nucleated cells causing false positive diagnoses is recognized and solutions are being studied. The data base on static slit-scan contours is being continually expanded.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The development of a flow-through screening system for cervical cytology will allow greater accuracy, speed and availability in screening and diagnosis of cancerous and pre-cancerous lesions.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: November 8, 1972
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 74 Funds: $286,354

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigators: Name/Address Performing Organization:	Walter E. Tolles, Ph.D. SUNY - Downstate Medical Center Brooklyn, New York 11203

Contract Number:
Starting Date: Expiration Date:
Goal: Evaluation of multiparameter cell sorter designed for cytology automation.

Approach: The Contractor shall deliver to the Laboratory of Pathology 775 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: New contract pending negotiation .

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date:
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY75 Funds: $24,000

CONTRACT RESEARCH SUMMARY

Title: A Pre-clinical Evaluation of ^{67}Ga as a Marker for the Automated Detection of Neoplasia of the Uterine Cervix.

Principal Investigator: Name/Address Performing Organization:	Carolus Cobb, Ph.D. American Science and Engineering, Inc. 955 Massachusetts Avenue Cambridge, Massachusetts, 02139

Contract Number:
Starting Date: Expiration Date:
Goal: Determination of the cell types from human uterine cervix showing an increase affinity for ^{67}Ga.

Approach: The contractor will study cells prepared with the AS&E technique for inducing differential uptake for a possible use of ^{67}Ga as a marker for cytology automation. Cell types investigated will be normal, pre-malignant and malignant human vaginal-cervical cells. Study of sub-cellular localization of the increased ^{67}Ga uptake will also be performed.

Progress: New contract pending negotiation.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Investigation of differential uptake of ^{67}Ga for a possible use of ^{67}Ga as a marker for distinguishing neoplastic from non-neoplastic human cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date:
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY75 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Supply Cytoscreener and Technician

Principal Investigator: Name/Address Performing Organization:	Melvin P. Ehrlich Nuclear Leasing Corporation P. O. Box 3511 Hyde Park, N.Y. 11040

Contract Number:
Starting Date: Expiration Date:
Goal: To supply Cytoscreener for engineering and biological evaluation.

Approach: The Cytoscreener, manufactured and patented by Nuclear Research Associates, has been proposed as an instrument suitable for automation of clinical cytologic screening. Clinical tests have been equivocal. Basic engineering and biologic data are needed before further clinical testing can be conducted.

Progress: New Contract pending negotiation.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date:
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY75 Funds: 170,000

CONTRACT RESEARCH SUMMARY

Title: Investigation of High Resolution Fluorescence Measurements on Cells from the Human Female Genital Tract and Quantitative Study of Acridine Orange and other Fluorochromes.

Principal Investigator: Name/Address Performing Organization:	Leon L. Wheeless, Jr., Ph.D. University of Rochester Rochester, New York 14642

Contract Number:

Starting Date: Expiration Date:

Goal: Automation of clinical cytologic screening and diagnosis

Approach: The Principal Investigator will investigate "high resolution" fluorescence measurements on cells from the human female genital tract. A quantitative study of Acridine Orange and other fluorochromes useful in automated cell analysis systems will be performed.

Progress: New contract pending negotiation.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy

Program: Cytology Automation Site Visit Date:

Technical Review Group: Committee on Cytology Automation

Relevance Review Group: Cancer Diagnosis Steering Committee

FY75 Funds: $256,500

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Cytoscreener

Principal Investigator: Thomas P. Grover, Ph.D.
Name/Address Utah Biomedical Test Laboratory
Performing 520 Wakara Way, Salt Lake City
Organization: Utah, 84111

Contract Number:
Starting Date: Expiration Date:
Goal: Engineering and biological evaluation of the operation of the Cytoscreener.

Approach: The Cytoscreener has been proposed as an instrument suitable for automation of clinical cytologic screening. Clinical tests of the instrument have been equivocal. Basic engineering and biologic data are needed before further clinical testing can be conducted.

Progress: New Contract pending negotiation.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date:
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY75 Funds: 123,000

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigators: Name/Address Performing Organization:	Frank J. Bepko, Jr., M.D. D.C. General Hospital Washington, D.C. 20003

Contract Number:

Starting Date: Expiration Date:

Goal: Evaluation of multiparameter cell sorter designed for cytology automation.

Approach: The Contractor shall deliver to the Laboratory of Pathology 240 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual used in a fully automated clinical instrument.

Progress: New contract pending negotiation.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessiblity of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary Cassidy

Program: Cytology Automation Site Visit Date:

Technical Review Group: Committee on Cytology Automation

Relevance Review Group: Cancer Diagnosis Steering Committee

FY75 Funds: $15,000

DATE DUE

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